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# Arm crank power and hyperammonemia in respones to L-Aspartic Acid supplementation

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# ARM CRANK POWER AND HYPERAMMONEMIA IN RESPONSE TO L-ASPARTIC ACID SUPPLEMENTATION

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# PREFACE

This dissertation was a sequence of six investigations conducted in two phases, as a collaboration between the Department of Kinesiology at Louisiana State University, Baton Rouge, Louisiana, and the Department(s) of Biology and Exercise and Sports Science, Brigham Young University – Hawaii, Laie, Hawaii. The first phase was a series of three pilot studies, while the second phase was three experiments. The presentation style is based on that adopted by Acta Physiologica Scandinavica for publication of medical dissertations (New Series No. 641 ISSN 0346-6612 ISBN 91-7191-748-9, Acta Physiologica Scandinavica Volume 168 Supplementum 646, January 2000). Chapter 1 provides a critical review of the literature, presentation of the problem, and discussion of the rationale for experimentation. Chapter(s) 2, 3, and 4, present each experiment, respectively. Chapter 5 presents overall discussion and conclusions, and suggestions for future work.

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ABSTRACT

PURPOSE: To examine the effect of L-Aspartic Acid (L-Asp) supplementation on short-term arm-crank power (i.e. $W \cdot min^{-1}$) and exercise-induced hyperammonemia in two gender-specific investigations. METHODS: The male investigation (MALE) used nine highly-trained intercollegiate water polo players (WP) and nine moderately-trained college students (MOD); the female investigation (FEM) used 18 recreationally-trained college students. All exercised to exhaustion on two occasions following counterbalanced double-blind ingestion of 12.5g x 2 of L-Asp or placebo (P). The warm-up phase began at 25 $W \cdot min^{-1}$ and increased in 25 $W \cdot min^{-1}$ stages every 30 seconds (constant 100 rpm). Performance began (i.e. the subjects turned the crank 'as fast as possible for as long as possible') with stage completion of rating of perceived exertion of 17 (6-20 scale) in MOD and FEM and at the 4-minute mark (200 $W \cdot min^{-1}$) for WP. Individual warm-up time in session 1 for MOD and FEM was used as warm-up time for session 2, respectively. Venous blood samples were drawn at 3 minutes post-exercise and assayed for blood $NH_3$ [μM/L]. A 2 x 2 ANOVA with repeated measures was used for MALE, and a one-way ANOVA with repeated measures for FEM, with Tukey's t-test for post hoc differences. RESULTS: Blood $NH_3$ [μM/L] decreased with L-Asp (vs. P) in WP ($241.0 \pm 18.4$ vs. $200.6 \pm 20.5$, $p < 0.01$) and MOD ($219.7 \pm 20.8$ vs. $185.6 \pm 27.5$, $p < 0.05$). Performance power ($W \cdot min^{-1}$) with L-Asp improved ($p < 0.01$) in WP ($270.2 \pm 19.7$ vs. $227.6 \pm 14.3$) but not ($p > 0.05$) in MOD ($220.1 \pm 9.1$ vs. $204.4 \pm 8.3$). There was no treatment effect in FEM. CONCLUSION: These results suggest that 12.5 g x 2 QD of L-Asp reduces hyperammonemia and enhances short-term power in intercollegiate water polo players. Conversely, in MOD and FEM, 12.5 g x 2 QD of L-Asp does not attenuate exercise-induced hyperammonemia sufficiently to improve power.

# CHAPTER 1. REVIEW OF LITERATURE

## 1.1 Introduction

Ammonia ($NH_3$) is a normal metabolic product. At high physiological levels, however, $NH_3$ is adverse to clinical status, metabolic function, and exercise performance. Blood ammonia concentrations are normally < 50 μM/L at rest (Fischbach, 1988). However, as reviewed by Batshaw (1984), prolonged symptomatic blood concentrations exceeding 100 μM/L comprise the medical condition known as clinical hyperammonemia (CH). The symptomatic etiology of CH is encephalopathy preceded by hepatic dysfunction (Allen and Conn, 1960; Bessman and Bradley, 1955). Strenuous physical activity elevates blood ammonia concentrations to the criteria for mild to moderate CH (100 μM/L – 500 μM/L). Interestingly, the symptoms and blood ammonia values of mild to moderate CH are similar to those observed during physical exhaustion with 'exercise-induced hyperammonemia' (EIH). These include confusion, hyperventilation, and muscular dysfunction. If prolonged, CH symptoms may exacerbate to convulsions, coma, and death. With EIH, maintenance of high power is difficult, and exhaustion is imminent (Allen and Conn, 1960; Banister et al., 1983; Hermansen and Stensvold, 1972; Wilkerson et al., 1977). Treatments for lowering CH and EIH are similar, and include: 1) restriction of dietary protein, 2) increasing carbohydrate (%) intake, 3) administration of amino acids (AA's) that enhance ammonia clearance into (the less toxic) urea, and 4) correction of the underlying metabolic etiology. A major focus of sports nutrition physiology is performance improvement by enhancing ATP production, glycogen storage, and sports-specific training. As reviewed by Graham et al. (1997), Yuan and Chan (2000), and noted by Brooks et al. (1996), "amino acids and proteins are, in fact, integrally involved in the metabolic adjustment to exercise". Although understood that lower blood ammonia levels are desirable for optimal performance, few report the impact of acutely reducing EIH by

supplementation with ammonia-reducing amino acids (e.g. L-arginine, L-ornithine, L-aspartate). Further, the impact of lowering blood ammonia in this manner to improve short-term high-intensity performance is unknown. Therefore, the primary focus of this dissertation is to critically address the importance of metabolic ammonia and the impact of reducing ammonia on exercise performance.

As reviewed by Graham et al. (1997), there is an apparent beneficial association between exercise performance, reduced blood ammonia concentration, and improved training status. For instance, Lo and Dudley (1987) reported that endurance trained subjects had lower plasma ammonia levels than untrained subjects at the same absolute power output during a short-term incremental protocol. Data from Snow et al. (1992) demonstrated the benefits of seven weeks of sprint training include absolute increases in power and reduced blood ammonia at a given relative exercise intensity. As suggested by Holloszy and Coyle (1984) and demonstrated by Denis et al. (1989) and Green et al. (1991), short-term endurance training lowers blood ammonia at a submaximal intensity. However, as cited by Graham et al. (1997), it is doubtful that two weeks of endurance training (Denis et al., 1989) is sufficient to increase mitochondrial density, plus, sprint training should not tax the mitochondria. Therefore, there must be some other mechanism(s) (in an overall or combined fashion) for the reduction of blood ammonia with training. To date, it is unclear whether the blood ammonia response to training reflects reduced muscular production, greater removal from the blood, enhanced buffering in either muscle or blood, or increased conversion into amino acid(s) (i.e. branched chain amino acids).

As described by Mutch and Banister (1983), fatigue is the decreased ability of an organism or one of its parts to respond or function because of prolonged exertion or repeated stimulation. Although muscular fatigue is a well-known phenomenon and

"exercised until exhaustion" is understood, a myriad of factors contribute to fatigue. These include:

1) Substrate depletion of adenosine triphosphate (ATP), phosphocreatine (PC), and carbohydrate (CHO),
2) Accumulation of waste products (e.g. $CO_2$, $H^+$, $NH_3$, hypoxia),
3) Inhibition of metabolic function, and/or
4) Central factors (e.g. tryptophan uptake by the brain, leading to seratonin accumulation).

For example, Frumerie (1913) pointed out that the onset of physical fatigue is experienced initially at the joints and tendons, while Mosso (1915) suggested that fatigue was a consequence of "a kind of poisoning resulting from products derived from chemical changes in cells". The hydrogen ion hypothesis (i.e. muscular fatigue as a result of tissue acidosis) was first proposed by Hill et al. (1924), who noticed a consistent relationship between the rate of recovery from a tetanic contraction and the rate of disappearance of lactic acid from muscle.

At rest, circulating ammonia exists in minute quantities [10 - 40 μM/L] and is tolerated without symptoms. However, prolonged clinical hyperammonemia ([100 - 2000 μM/L]) interferes with respiration, cell and mitochondrial permeability, and central nervous system (CNS) function (Batshaw, 1984). Deterioration of brain function in response to hyperammonemia is progressive, and if exacerbated, is often fatal. As reviewed by Mutch and Banister (1983), ammonia is produced both centrally and peripherally, and depletes reserves of glutamate, aspartate, and GABA, essential neurotransmitters. Further, Mutch and Banister (1983), and Banister et al. (1985) have suggested that the accumulation of ammonia is a contributing factor to muscular exhaustion. It is well known that strenuous muscular activity elevates blood ammonia to the symptomatic levels observed in clinical hyperammonemia (> [100 μM/L]) (Allen and Conn, 1960; Banister et al., 1983; Hermansen and Stensvold, 1972; Wilkerson et al., 1977). Data from Eriksson et al. (1985) and Graham

et al. (1990) suggest that the blood ammonia rise with exercise reflects production rate exceeding removal. Further, Washio and Mahima (1963) and Heald et al. (1975) demonstrated progressively inhibited in situ contractile responses of skeletal muscle in concentrated extracellular ammonium ion solutions. Washio and Mashima (1963) demonstrated that the majority of muscle fibers (e.g., 22 of 25) become electrically inexcitable following progressive exposure to ammonium ions. These observations suggest that 1) muscular performance and ammonia levels are inversely related, and 2) excessive ammonia accumulation is a regulator of performance.

Fortunately, acute and chronic reductions of hyperammonemia generally improve clinical prognosis and muscular performance (Batshaw, 1984). In apparently healthy populations, the magnitude of EIH reduction to improve performance is not established, nor are target population(s) and methods (e.g. exercise modalities, intensities, and duration). In the clinical setting, the amino acids L-Aspartate, L-Ornithine, L-Arginine, and/or L-Glutamate reduce CH and increase desirable prognosis (Balesteiri et al., 1967; DuRuisseau et al., 1956; Najarian et al., 1958; Winitz et al., 1956). However, exercise studies using similar supplementation (Denis et al., 1991; Edwards and Day, 2000; and Eto et al., 1994) indicate that L-Ornithine, L-Arginine, and/or L-Glutamate are ineffective as an ergogenic aid for the acute reduction of EIH and/or improvement in exercise performance.

Alternatively, data from animal and human studies suggests that in certain situations administration of L-Aspartic Acid, (or one of the magnesium-potassium salts of L-Aspartate) lowers blood ammonia concentration (Kendrick, 1976; Keul, 1971; Laborit et al., 1958; Wesson et al., 1988) and enhances performance (Ahlborg et al., 1968; Franz and Chintanaseri, 1977; Gupta and Srivastava, 1973). Application of the results is controversial, possibly due to the variability in training status of testing populations, dosage regimes, and modalities. Although data from Derave et al. (1997) demonstrate gender

differences in ammonia response, the effect of aspartate supplementation on exercise performance in women is unknown. The impact of aspartate ingestion to reduce transient EIH and enhance short-term high-intensity (> 90% $VO_{2max}$) power when blood ammonia levels peak, has not been reported. Therefore, this critical review and dissertation will focus on the bioenergetics and impact of L-Aspartic Acid supplementation to reduce EIH and improve high-intensity exercise performance in men and women.

## 1.2 Bioenergetics: ATP Production, $NH_3$ Production, $NH_3$ Removal

### 1.2.1 Introduction

The high-energy compound ATP (Adenosine Triphosphate), derived from foodstuffs, provides essentially all of the energy required for physiological functions. However, due to a low amount of ATP readily available in cells, specific metabolic pathways for digested components (ATP-PC, carbohydrate, amino acids, triacylglycerol derivatives) produce ATP for energy. As discussed by Astrand and Rodahl (1986), recruitment of substrate for ATP production is mediated by:

1) Exercise intensity, duration, modality, type (continuous or intermittent)
2) State of training
3) Diet
4) State of health
5) Availability and source of substrate.

Table 1.1 lists the various biochemical pathways, energy sources, production characteristics, and waste products of ATP metabolism, assuming 10 kcal/mole ATP (Brooks et al., 1996). Muscular activity increases ATP utilization rate, demand for replenishment, and waste product formation (e.g. $NH_3$, $H^+$, $CO_2$). The waste products generated depend on the source, speed, and amount of substrate consumed. Ammonia, for instance, accumulates rapidly with 1) accelerated ATP hydrolysis (i.e. during short bursts of high-intensity activity) and/or 2) inhibited removal (e.g., limitation(s) in urea cycle enzyme efficiency, urea cycle substrate availability, and hepatic blood flow). As

Table 1.1. ATP sources, production characteristics, and waste products.

| **Metabolic Pathway(s)** | **Energy Source** | **Estimated Rate (moles/min)** | **Estimated Capacity (moles)** | **Waste Products** |
|---|---|---|---|---|
| ATP-PC | Intramuscular ATP Phosphocre-atine | Very Fast (> 3.6 ATP/min) | Very Low (0.5 ATP) | $P_i$ $NH_3$ |
| Glycolysis (exclusively) | Glucose Glycogen | Fast (1.5 ATP/min) | Low (500g CHO – 9 ATP) | $H^+$ |
| Glycolysis + TCA + ETC | Glucose Glycogen | Somewhat Fast (1 ATP/min) | Med (500g CHO - 114 ATP) | $CO_2$ |
| Gluconeogenesis; Transamination/ Oxidation – TCA Cycle, ETC | BCAA's Glycerol Lactate Alanine | Variable | High (2500g PRO-175 ATP) | $NH_3$ $CO_2$ |
| β-Oxidation -TCA Cycle, ETC | Fatty acids Glycerol Ketones | Very Slow | Very High (>15,000g Fat – 8,000 ATP) | $CO_2$ |

depicted in Table 1.1, maximal ammonia production occurs in short term high-intensity athletic events (e.g. 400 – 800 meter run, water polo sprints), which accelerate ATPase, adenylate kinase, and adenosine monophosphate (AMP) deaminase activity (Astrand and Rodahl, 1986). During intense exercise, a 50% reduction in muscular [ATP] yields up to a 20-fold change in adenosine diphosphate [ADP], and 700-fold increase in [AMP] (Astrand and Rodahl, 1986). Elevated blood $NH_3$ occurs also with long duration exercise, but to a smaller degree, from the metabolism of branched chain amino acids (BCAA's) used for gluconeogenesis. The amount of ammonia produced with endurance exercise is far greater

(and more tolerable) versus short-term high-intensity exercise, due to a lower rate of production (Graham et al., 1995a; Wagenmakers et al., 1990). The demand for ATP, availability of substrate, and status of training are principal factors in ammonia metabolism. The bioenergetic sources, fates, and impact of ATP, ammonia, and aspartate are discussed in the following sections of this chapter.

### 1.2.2 ATP - PC System

ATP is a readily available energy source in active muscle, consisting of a base (adenine), a sugar (ribose), and a chain of three phosphates. During short-term intense exercise, ATP depletion (i.e. demand exceeding supply) occurs rapidly (i.e. < 1 minute). In response, additional pathways are activated to 1) reproduce ATP quickly (i.e. glycolysis, gluconeogenesis), and 2) transport and remove waste products (i.e. $NH_3$) efficiently (e.g. transamination, Cori Cycle activity, urea cycle activity,). Ammonia is a deamination by-product of the very fast phosphagen (ATP-PC) pathway for ATP production, utilization, and regeneration (Sabina et al., 1984). ATP hydrolysis elevates ADP, AMP, inosine monophosphate (IMP), and ammonia via coupling of ATPase, AMP deaminase, and the purine nucleotide cycle (PNC) (Hellsten et al., 1999). The impact of ATP hydrolysis, the PNC, and ammonia production on bioenergetics are presented and discussed in this chapter.

Schmidt (1928) first reported that AMP deamination, in response to accelerated ATP hydrolysis, is a major source of ammonia production in active muscle. As presented below in reactions (1) and (2), the ATP-PC pathway is a 2-reaction cycle. Accelerated in response to high-intensity exercise, the products of the ATPase (1) (ADP, Pi) become reactants for ATP regeneration either by either a reversal of reaction (1) (utilizing energy released); or by myokinase in reaction (2) (ADP + ADP <=> ATP + AMP). The production of $NH_3$ by AMP deaminase (AMP + $H_2O$ => IMP + $NH_3$), is the primary source

of the $NH_3$ (reaction (3)) rise with short-term intense activity (Sabina et al., 1984; Schmidt, 1928; Wajzer et al., 1956). Studies by Meyer and Terjung (1979; 1980) have shown a 50% conversion rate of ATP into IMP and ammonia during short-term intermittent stimulation (5 min) of rat gastrocnemius muscle.

$ATP + H_2O <=> ADP + P_i$ (ATPase) (1)
$ADP + ADP <=> AMP + ATP$ (myokinase) (2)
$AMP + H_2O => IMP + NH_3$ (AMP deaminase) (3)

**1.2.3 Purine Nucleotide Cycle**

Ammonia production via AMP deaminase is proportional to the rate of ATP hydrolysis. Although AMP deaminase ($AMP + H_2O => IMP + NH_3$) is irreversible, AMP may be regenerated (reactions 4,5) by a process termed the purine nucleotide cycle (PNC) (Goodman and Lowenstein, 1977; Lowenstein, 1972; Wheeler and Lowenstein, 1979). According to Banister et al. (1983), maintenance of the total amount (TAN) of adenine nucleotides (ATP + ADP + AMP) during exercise is desirable. Further, the PNC requires aspartate as a substrate (reaction 4). Thus, aspartate administration stimulates the regeneration of AMP assisting the maintenance of TAN's. In addition, the TCA cycle intermediate fumarate (reaction 5) is a product of the PNC. The PNC steps are:

$AMP + H_2O => IMP + NH_3$ (AMP deaminase) (3)
$IMP + Aspartate + GTP => Adenylosuccinate + GDP + P_i$ (adenylosuccinate synthetase) (4)
$Adenylosuccinate <= > AMP + Fumarate$ (adenylosuccinase) (5)

The overall summary is:

$Aspartate + GTP + H_2O => Fumarate + NH_3 + GDP + P_i$ (6)

The bioenergetic results of the PNC are:

1) Regeneration of AMP
2) Deamination of aspartate to fumarate, and
3) Liberation of ammonia.

Data from several studies (Gerez and Kirsten, 1965, Meyer and Terjung, 1979; Raggi et al., 1969; Winder et al. 1974) indicates that recruitment of fast-twitch muscle

fibers (vs. slow twitch) increases AMP deaminase activity and ammonia production. AMP deaminase is activated also by high intramuscular [ADP], [AMP], and a fall in pH -- and inhibited by high [ATP], [GTP], and [$P_i$] (Wheeler and Lowenstein, 1979). On the other hand, slow-twitch muscle fibers contain low amounts of AMP deaminase (Gerez and Kirsten, 1965; Meyer and Terjung, 1979; Raggi et al., 1969; Winder et al. 1974), and therefore produce less ammonia. Thus, mitochondria-rich muscle (slow oxidative) produces less ammonia with high-intensity exercise than mitochondria-poor (fast glycolytic) muscle. Washio and Mashima (1963), and Heald (1975) demonstrated inhibited in situ skeletal muscle contractile responses in a concentrated extracellular ammonium ion solution. Therefore, recruitment of fast-twitch muscle fibers accelerates ATP hydrolysis, AMP deamination, PNC activity, and intramuscular [$NH_3$].

Summarizing, the resynthesis of AMP by the PNC requires aspartate for the maintenance of total adenine nucleotides. Secondly, the PNC and aspartate provide carbons for fumarate for TCA cycle bioenergetics (Lowenstein, 1972). The impact of removing (or buffering) the toxic ammonia product is a priority, and will be discussed in detail in the section on ammonia removal.

### 1.2.4 Glycolysis and Pyruvate Oxidation

Second in speed to the ATP-PC system for ATP production is glycolysis. As discussed by Miller (1992), glycolysis is the 10-reaction catabolism of the 6-C glucose in the cytosol into two 3-C molecules of pyruvate. Glycolysis generates a net of two ATP (i.e. 4 ATP created, minus 2 ATP utilized). Glycolysis is a process of energy transfers where the phosphate is carried from substrate to ADP to ATP via the phosphate ion itself, and thus, is termed 'substrate-level phosphorylation'. Glycolysis does not require oxygen. Two NADH molecules are produced, which result in six additional ATP produced by the electron transport chain (ETC). Intense muscular activity (e.g. where exhaustion occurs in

less than 2 minutes) accelerates glycolytic rate and produces (and utilizes) a relatively large amount of ATP in a short period. As a potent activator of the glycolytic rate-limiting enzyme phosphofructokinase (PFK), elevated [$NH_3$] concentrations accelerate glycogen depletion, ATP production, and ATP hydrolysis (Felig and Wahren, 1971; Lowenstein, 1972; Sugden and Newsholme, 1975).

Pyruvate metabolism is dependent upon ATP supply and demand. When conditions favor pyruvate oxidation (e.g. during moderate continuous physical activity), pyruvate is converted into acetyl-CoA by the pyruvate dehydrogenase complex (PDH) within the mitochondria. The PDH complex is a series of the three enzymes E1 (pyruvate dehydrogenase), E2 (lipoate acyltransferase), and E3 (dihydrolipoyl dehydrogenase). Also termed 'oxidative decarboxylation', the PDH sequence is important in highly oxidative tissue such as muscle and heart, by promoting pyruvate conversion into acetyl-CoA for entry into the TCA cycle. The PDH complex converts the 3-C pyruvate molecule to 2-C acetyl-CoA, NADH, releasing $CO_2$, as summarized as follows:

$$\text{Pyruvate} + NAD^+ + \text{CoASH} \xleftrightarrow{\text{PDH complex}} \text{Acetyl-CoA} + \text{NADH} + H^+ + CO_2$$

Advantages of pyruvate decarboxylation include 1) more ATP produced per unit glucose and 2) reduced lactate, $H^+$, and ammonia accumulation. However, a noteworthy disadvantage is the slower rate of ATP production with pyruvate oxidation.

On the other hand, when ATP needs are accelerated during short bursts of intense activity, hydrogens accumulate and combine with pyruvate. In this case, lactate and $NAD^+$ form rapidly, due to the near equilibrium of lactate dehydrogenase (LDH) reaction. Lactate may be converted back (i.e. gluconeogenesis) into 1) glucose in the liver, or 2) pyruvate by the heart or oxidative fibers (Broberg and Sahlin, 1988). Advantages of lactate production include 1) faster rate of ATP production and 2) the recycling of $NAD^+$ into NADH for

anaerobic production of ATP to continue, and 3) providing $NAD^+$ to accept additional hydrogens generated in glycolysis. However, the disadvantages of accelerated lactate formation include: 1) very fast depletion of a very limited supply (~ 500g) of carbohydrate, and 2) rapid accumulation of lactic acid, $H^+$ and subsequent ammonia from ATP hydrolysis – each inhibiting muscular contraction and promoting fatigue. Therefore, the responses to accelerated glycolytic fiber activity include 1) faster production and utilization of ATP and NADH, 2) elevated lactate and $H^+$ production, 3) elevated ammonia (from ATP hydrolysis), and 4) excitatory feedback activation of PFK by ammonia (Felig and Wahren, 1971; Lowenstein, 1972; Sugden and Newsholme, 1975).

### 1.2.5 Tricarboxylic Acid (TCA) Cycle

The primary purpose of the TCA cycle is to provide high energy reducing equivalents (NADH, $FADH_2$) for the electron transport chain for ATP production within the mitochondria. Acetyl-CoA is the common entry point into the TCA cycle for carbohydrate, fat, and protein catabolism. In this process, the 4-C TCA intermediate oxaloacetate (OAA) condenses with the 2-C acetyl-CoA to yield the 6-C TCA intermediate citrate. Summarizing, the TCA cycle strips the hydrogens and carbons from the acetyl Co-A shell, producing:

1) 2 molecules of $CO_2$
2) 1 ATP equivalent
3) Eight hydrogen atoms in the form(s) of NADH and $FADH_2$, carrying high energy transfer potential to the electron transport chain for $NAD^+$ and ATP production.

### 1.2.6 Electron Transport Chain

In 1961, a paper by Peter Mitchell introduced the chemiosmotic hypothesis (i.e. 'the electron transport chain') for the production of ATP within the mitochondria (Mitchell, 1961). The electron transport chain (ETC) is the process of ATP production within the mitochondria utilizing the high-energy potential of the electrons of the hydrogen atoms in

the transfer to molecular oxygen. The reducing equivalents NADH and $FADH_2$ (produced in various metabolic pathways) transfer this high-energy potential (Miller, 1992) across the inner mitochondrial membrane. As described below, the energy transfers promote ATP production at three 'sites', utilizing specific electron carriers, respectively. Each site phosphorylates ADP into ATP (i.e. 'oxidative phosphorylation'), liberating a large amount of energy.

The passage of electrons to the final carrier of oxygen occurs at three sites with ATP produced at each. ADP is phosphorylated into ATP by electrical gradients created by the pumping of $H^+$ across the inner mitochondrial wall and cristae through the ATPases. NADH electrons enter the ETC at site #1, while that of $FADH_2$ enter at site #2. At site #3, the electrons of NADH and $FADH_2$ both combine with oxygen to produce ATP and $H_2O$. The remaining energy is released as heat. Three ATP are produced for each NADH, while two ATP are produced with each $FADH_2$. The electron source(s) and transfer characteristics for each site are:

Site #1 - NADH electrons are transferred to coenzyme Q by NADH dehydrogenase (producing 1 ATP)
Site #2 - NADH and $FADH_2$ electrons are transferred from coenzyme Q, to cytochrome b, to cytochrome $c_1$ (producing a second ATP)
Site #3 - NADH and $FADH_2$ electrons are transferred from cytochrome $c_1$, to cytochromes c, a, $a_3$, to oxygen (producing a third ATP, and water)

The energy released during the transfers promotes the phosphorylation of ADP into ATP, producing $H_2O$, and releasing heat. Oxygen is the final electron acceptor. The effects of $NH_3$ on the coupling of electron transport, proton gradients, and ATP production are unknown. However, as discussed in the following paragraph, ammonia and aspartate fluctuations may affect the malate-aspartate shuttle for reducing equivalent transport in the ETC.

The inner mitochondrial membrane is impermeable to NADH. To participate in the ETC, NADH is shuttled across the membrane to site #1 or site #2 by one of two mechanisms: 1) the malate aspartate shuttle and/or 2) the glycerol phosphate shuttle (Miller, 1992). Site #1 of the ETC utilizes the malate aspartate shuttle to transfer electrons from NADH to $NAD^+$ resulting in 38 ATP from one glucose molecule. Site #2 utilizes the glycerol phosphate shuttle to transfer electrons from NADH to $NAD^+$, resulting in 36 ATP from one glucose molecule. Since the site using the aspartate shuttle produces more ATP per glucose molecule, it appears advantageous for bioenergetics to have more aspartate available for the ETC.

Therefore, as discussed by Miller (1992) aspartate availability may enhance ETC productivity by:

1) Promoting the shuttling of reducing equivalents across the inner mitochondrial membrane,
2) Producing 2 additional ATP via ETC site #1 versus site #2, and
3) Providing substrate for the PNC for the maintenance of total adenine nucleotides.

### 1.2.7 β-Oxidation

The conversion of fat (triacylglycerol) for ATP integrates several metabolic pathways, and culminates in the splitting of the fatty acid molecule into 2-C acetyl fragments, NADH and $FADH_2$ for utilization by the TCA cycle and the ETC (McArdle et al., 1996). Although fatty acid oxidation is slower than glycolysis, the relative (and absolute) amount of ATP produced from it is much higher (~130 ATP per 16-C palmitate) than glycolysis. Ammonia is not a product of β-oxidation, nor are fatty acids a substrate for glucose synthesis. However, glucose and fatty acid oxidation link ATP production. For example, a prolonged shortage of the 4-C TCA intermediate OAA results in 1) undesirable ketone (acetone, acetoacetate, β-hydroxybutyrate) formation from excess acetyl-CoA buildup, and 2) decreased OAA for gluconeogenesis TCA continuity. As described by

Brooks et al. (1996), the sequence for lipid catabolism for ATP production (beginning with triacylglycerol cleavage) is as follows:

1) Mobilization - initiation of exercise triggers a release of epinephrine, promotes triacylglycerol breakdown (by hormone sensitive lipases, HSL) into three fatty acids and a glycerol backbone, per triacylglycerol. Glycerol may contribute carbons for glucose synthesis, or enter glycolysis as 3-phosphoglyceraldehyde. The complete breakdown of glycerol yields 19 ATP, synthesized from substrate level phosphorylation and release of hydrogen atoms to $NAD^+$ with subsequent pyruvate oxidation
2) Circulation - free fatty acid (FFA) transport via the blood to muscle (usually with albumin as the carrier)
3) Uptake - the entry of free fatty acids (FFA) into muscle
4) Activation - resembling the first step in glycolysis, ATP and coenzyme A activate the fatty acid in the cytoplasm, forming fatty acyl-CoA for transport into the mitochondria
5) Translocation - carnitine shuttles the fatty acyl-CoA into the inner mitochondria
6) β-oxidation - a cycle where the carbons of the activated fatty acid chain (e.g, palmitate, 16-C) are stripped away two at a time (between the first carbon from the carboxyl end - the α; and the second carbon - the β) producing a 2-C acetyl-CoA, NADH, and $FADH_2$ per turn of the cycle. This process is repeated until the entire carbon chain of the fatty acyl-CoA is utilized. The final spin of the cycle (when only 2 C are left) produces acetyl-CoA only. Combining subsequent mitochondrial oxidation (TCA cycle and ETC), each acetyl-CoA yields 12 ATP (TCA and ETC); each NADH yields 3 ATP (ETC); and each $FADH_2$ yields 2 ATP (ETC). The total amount of ATP produced, depends on the number of carbons in the fatty acid.
7) Mitochondrial Oxidation - the TCA cycle and ETC produce ATP from acetyl-CoA, NADH, and $FADH_2$. For example, the complete oxidation of a 16-C palmitate FA yields a net total of 130 ATP [(12 x 8 = 96 ATP from acetyl-CoA) + (7 x 3 = 21 ATP from NADH) + (7 x 2 = 14 ATP from $FADH_2$) - 1 ATP invested for activation], or approximately 390 ATP per palmitic triglyceride (exclusive of glycerol).

Thus, for a 16 carbon FA, the first seven cycles of β-oxidation yield:

1) 7 Acetyl-CoA (= 84 ATP),
2) 7 NADH (= 21 ATP), and
3) 7 $FADH_2$ (= 14 ATP).

The last revolution of β-oxidation yields one acetyl Co-A (= 12 ATP), producing a total of 131 ATP, (minus 1 ATP invested for FA activation), for a net of 130 ATP per molecule of palmitate.

### 1.2.8 Amino Acids: Digestion, Bioenergetics, Ammonia, and Aspartate

Carbohydrate, fat, and protein are the primary food sources for mammals with each providing carbons for energy (Miller, 1992). Of these, only proteins contain nitrogen, producing ammonia from catabolism. Proteins are polypeptide chains of the 20 different amino acids, arranged in any order, up to thousands of AA's long. AA's are composed of carbon, hydrogen, oxygen, nitrogen, and occasionally sulfur. Each AA has four components:

1) An amine group ($NH_2$),
2) A carboxyl group ($COOH^-$),
3) A unique side "R" chain, and
4) A hydrogen.

The amino group of each AA is attached to the α carbon (the carbon next to the carboxyl carbon) of a carboxylic acid (Brooks et al., 1996). Peptide bonds attach the amine group of one AA to the carboxyl group of another, forming long strands called proteins. Metabolism of proteins and AA's has several key bioenergetic characteristics, including:

1) No significant storage pool specific for energy, analogous to storage of glycogen or triacylglycerol
2) AA carbons are precursors for gluconeogenesis
3) AA's catabolize to produce other AA's
4) Substrate of anaplerosis for the TCA cycle (supply TCA intermediates)
5) Ammonia as a product.

The digestion of protein into AA's begins in the stomach. Here, pepsin (under the influence of HCl) splits the large protein molecules into smaller units, called polypeptides. Proteinases and peptidases degrade the polypeptides into individual AA's in the small intestine. AA's are absorbed primarily by the microvilli, and transported to the liver via the hepatic portal system. Data in laboratory animals from Neame and Wiseman (1956) and Finkelstein et al. (1983) indicate that portal absorption of aspartic acid, for example, begins about 10 minutes after introduction to the small intestine lumen, peaking at 30 minutes, and remaining above resting levels for 1-2 hours. During the absorptive state, AA's are utilized

for hepatic protein synthesis, and conversion into triacylglycerol. In contrast to the storage fate(s) for CHO (i.e. glycogen) and fat (i.e. triacylglycerol), there are no bioenergetic storage depot(s) specific for protein or AA. Fates of excess digestive protein are:

1) Conversion into triacylglycerol, and
2) Degradation into ammonia and urea by the liver, or
3) Degradation into ammonia by the intestinal flora by urease.

As pointed out by McArdle "all protein exists as important constituents of metabolic, transport, and hormonal systems" (McArdle et al., 1996). During exercise, glucagon, glucocorticoids, and catecholemines work together to catabolize proteins, particularly muscle proteins, for energy production. AA bioenergetic activity is promoted by:

1) Glucagon and catecholemines (e.g., a high protein, low carbohydrate diet)
2) Glucocorticoids (severe stress, e.g. burns, exercise, trauma, surgery, disease)
3) Low fuel reserves.

Glucagon (released from the pancreas) promotes hepatic glycogenolysis and blood glucose elevation. Catecholemines (released from the medulla of the adrenal glands), activate catabolism of stored substrate (e.g. lipolysis, glycogenolysis) for energy production. Glucocorticoids (released from the adrenal glands) cause proteins (e.g. actin, myosin) from muscles and connective tissue to be broken down into AA's to make glucose for energy. The bioenergetic effect(s) of glucagon and epinephrine are more immediate than glucocorticoids.

As illustrated below in reactions (1), (2), and (3) the two primary processes for stripping AA carbons for bioenergetics are transamination and oxidative deamination. Glutamate serves as a substrate (and nitrogen carrier) in all three reactions. In transamination, the amino group of an amino acid is transferred to a keto acid, thus producing a second amino acid and keto acid. The two major routes of transamination are glutamate-pyruvate transaminase (SGPT) (reaction 1), and SGOT (serum glutamate-OAA

transaminase, or aspartate aminotransferase) (reaction 2), each requiring pyridoxal phosphate as a coenzyme. Since transamination occurs in muscle and (in particular) the liver, both SGPT and SGOT are useful for analysis of bioenergetics and acute liver function. Mole' and Johnson (1971) have demonstrated elevated SGPT (also called alanine transaminase, or alanine aminotransferase, ALT) with endurance activity, activating alanine metabolism and production (reaction 1).

$$\text{Pyruvate (liver)} + \text{Glutamate} \xrightarrow{\text{SGPT}} \text{Alanine (muscle)} + \alpha\text{-ketoglutarate} \qquad (1)$$

As illustrated in reaction (2), the second transamination reaction, SGOT, links the amino acid(s) glutamate and aspartate with the TCA intermediates OAA and α-ketoglutarate. In addition to linking bioenergetics, aspartate is a major carrier of catabolic nitrogen to the urea cycle for excretion. The terminal nitrogen products of ammonia and urea leave the body via sweat, the lungs, and/or diluted in urine (Czarnowski et al., 1995).

$$\text{OAA} + \text{Glutamate} \xrightarrow{\text{SGOT}} \text{Aspartate} + \alpha\text{-ketoglutarate} \qquad (2)$$

Oxidative deamination occurs in the mitochondrial matrix, utilizing $NAD^+$ as the oxidizing agent. As illustrated in reaction (3), oxidative deamination assists the excretion of amino acid nitrogen from the body. Glutamate is converted by oxidative deamination to α-ketoglutarate and NADH, a reaction catalyzed by glutamate dehydrogenase (GDH). $NH_4^+$ serves as the nitrogen product. In amino acid catabolism, glutamate is a common nitrogen carrier, serving as an intermediate in both transamination (reactions 1 and 2) and oxidative deamination (reaction 3).

$$H_2O + \text{Glutamate} + NAD^+ \xrightarrow{\text{GDH}} \alpha\text{-ketoglutarate} + NH_4^+ + \text{NADH} \qquad (3)$$

Reactions 1 - 3 illustrate the concept of anaplerosis - how OAA, aspartate, glutamate, α-ketoglutarate, and ammonia link AA metabolism, gluconeogenesis, while regenerating TCA cycle intermediates. Aspartate is converted to OAA via transamination, i.e. transfers the amine group to glutamate while providing the carbon backbone for OAA. Aspartate is also converted to fumarate in the PNC, and the urea cycle, once again providing carbons for anaplerosis. Fumarate re-enters the TCA cycle, and is converted to OAA by TCA cycle enzymes. High ammonia levels drive the α-ketoglutarate GDH reaction (reaction 3) toward glutamate formation, thus depleting α-ketoglutarate, an essential TCA cycle intermediate.

AA carbon chains contribute to bioenergetics by providing the carbon backbone(s) for pyruvate, TCA cycle intermediates, or acetyl-CoA (and derivatives). AA's are classified by either function (intake need) or bioenergetic point of entry. The functional classifications are:

1) Nonessential – dispensable
2) Essential – indispensable (mammalian cells lack enzymes to synthesize the carbon skeletons)
3) Conditionally essential – essential under certain conditions (e.g. infancy)

The bioenergetic classifications are:

1) Glucogenic – AA carbon chains enter as pyruvate, or TCA cycle intermediate(s),
2) Ketogenic – AA carbon chains enter as acetyl-CoA or acetoacetate

The bioenergetic entry points for glucogenic AA's are:

1) Pyruvate - cysteine, alanine, glycine, serine
2) α-ketoglutarate - glutamate, glutamine, histidine, proline, arginine
3) Succinyl-CoA - isoleucine, methionine, threonine, valine
4) Fumarate - tyrosine, phenylalanine, aspartate
5) OAA - asparagine, aspartate

The bioenergetic entry points for ketogenic AA's are:

1) Acetyl-CoA - isoleucine, leucine, tryptophan
2) Acetoacetyl-CoA - phenylalanine, lysine, leucine, tyrosine

Therefore, tyrosine, phenylalanine, and isoleucine are classified both glucogenic and ketogenic, because their carbons may enter bioenergetics in either category.

FA's yield exclusively acetyl-CoA in β-oxidation; therefore, ketogenic AA (e.g., leucine) carbons are not a source of carbohydrate for gluconeogenesis. Gluconeogenesis does not result from the carbons of ketogenic AA's because: 1) the irreversible nature of PDH prevents pyruvate formation from acetyl-CoA, and 2) the 2-C atoms of acetyl-CoA are released as 2 molecules of $CO_2$ in the TCA cycle, and thus are eliminated. However, the catabolism of ketogenic AA's also produces NADH and $FADH_2$, thus providing high energy reducing equivalents for ATP production via gluconeogenesis and the ETC.

Fasting, starvation, and/or prolonged exercise deplete glycogen and blood glucose levels. In response to hypoglycemia, the body synthesizes new glucose (gluconeogenesis) via lactate, glycerol and AA's. Data from Mole and Johnson (1971), and Jungas et al. (1992), indicate that ~ 60% of the ATP produced from gluconeogenesis occurs in the liver, 35% from AA oxidation in muscle and small intestine, and ~ 5% from oxidation of acetoacetate.

Gluconeogenesis is a reversal of glycolysis, producing glucose as the end product of pyruvate catabolism. The process is costly, requiring six ATP for the conversion of pyruvate to glucose (compared to two ATP for glycolysis, and one ATP for β-oxidation) (Miller, 1992). The three irreversible glycolytic enzyme(s) of pyruvate kinase, PFK, and hexokinase are bypassed and substituted for in gluconeogenesis.

To bypass pyruvate kinase in gluconeogenesis, pyruvate is converted to phosphoenolpyruvate in a two-reaction process, with the 4-C TCA intermediate OAA serves as the intermediate. In the first reaction, pyruvate is converted to OAA by pyruvate carboxylase (PCB), driven by ATP and the cofactor biotin.

$$\text{Pyruvate} + CO_2 + \text{ATP} \xleftrightarrow[\text{Biotin}]{\text{PCB}} \text{OAA} + \text{ADP} + \text{Pi} \qquad (1)$$

In the second reaction, OAA is converted to phosphoenolpyruvate (PEP) via PEPCK, driven by GTP:

$$\text{OAA} + \text{GTP} \xleftrightarrow{\text{PEPCK}} \text{PEP} + CO_2 + \text{GDP} \qquad (2)$$

Gluconeogenesis then proceeds as a reversal of glycolysis, in the process bypassing two more irreversible glycolytic enzymes: 1) PFK by fructose 1-6 diphosphatase, and 2) hexokinase by glucose 6-phosphatase, eventually forming glucose as the product of gluconeogenesis.

Summarizing, beginning with pyruvate, gluconeogenesis is a reversal of the reversible glycolytic reactions, bypassing the highly exergonic glycolytic reactions of:

1) Pyruvate kinase (via pyruvate carboxylase and PEPCK);
2) PFK (via fructose 1-6 diphosphatase), and
3) Hexokinase (via glucose 6-phosphatase).

Gluconeogenesis is activated during impaired glucose availability, and/or insufficient ATP production (by the TCA cycle and the ETC) to meet demand. Important gluconeogenesis products (nitrogen and/or carbon) include ammonia, alanine, pyruvate, lactate, OAA, glucose, and ATP. As noted by Stryer (1995), accumulation of acetyl-CoA and pyruvate shifts bioenergetics in the direction of OAA formation and gluconeogenesis, by the action of pyruvate carboxylase. Further, data in animal models (Lancha et al., 1995) suggests that training status affects pyruvate carboxylase activity. For example, in response to high-intensity exercise, pyruvate carboxylase activity is higher in untrained (12x vs. rest) rat soleus versus trained (3x vs. rest). This suggests a glycogen sparing effect with training, in contrast to a greater rate of gluconeogenesis (versus β-oxidation) with

reduced training. Therefore, lesser-trained subjects exhibit higher exercising blood ammonia values, due in part to accelerated activity of BCAA catabolism for gluconeogenesis.

The branched chain AA's leucine, valine, isoleucine constitute a large percentage (20 - 30%) of dietary protein. During exercise, BCAA's are the primary AA's recruited for oxidizable substrate, and are catabolized more readily in peripheral tissues such as skeletal muscle, heart, adipose tissue, and kidney. On the other hand, human intestine and the liver have low levels of BCAA transaminase activity and BCAA keto acid dehydrogenase complex (Taniguchi et al., 1996).

Breakdown of BCAA's results in additional substrate (ketogenic and glucogenic) for energy, and as such, results in comparatively higher blood ammonia levels (Darmaun and Dechelotte, 1991) versus that say, of glycolysis or β-oxidation, because of the metabolism of the nitrogen skeleton. Working muscle releases the nitrogen component primarily in the form(s) of the amino acid(s) glutamine and alanine. BCAA catabolism begins with the transamination of the BCAA (leucine, valine, isoleucine) reacting with α-ketoglutarate, producing the following bioenergetic branched chain keto acid(s):

1) HMG-CoA, then acetoacetate and acetyl-CoA (via leucine)
2) Propionyl-CoA, then succinyl-CoA (via valine)
3) Propionyl-CoA and acetyl-CoA (via isoleucine)

BCAA catabolism also yield(s) NADH and $FADH_2$ via gluconeogenesis and the TCA cycle, thus providing high energy reducing equivalents for the ETC and ATP production. However, catabolism of the purely ketogenic BCAA leucine yields 2 carbon atoms (as acetyl-CoA), which are eventually released as $CO_2$ in the TCA cycle. Valine and isoleucine are both precursors to propionyl-CoA (a substrate for the TCA intermediate succinyl-CoA) and thus enter gluconeogenesis via OAA. However, catabolism of isoleucine yields both propionyl-CoA and acetyl-CoA, and therefore is both ketogenic and

glucogenic. Valine, yielding exclusively succinyl-CoA as a product, is purely glucogenic. Ketogenic AA's are not a source of glucose via gluconeogenesis for two reasons: 1) the irreversible nature of PDH prevents pyruvate formation from acetyl-CoA, and 2) the 2-C atoms of acetyl-CoA are released as two $CO_2$ in the TCA cycle, thus leaving no remaining carbons for gluconeogenesis.

The transfer of carbons between alanine and pyruvate between muscle and liver is termed the glucose-alanine Cori Cycle. The Cori Cycle links gluconeogenesis, alanine/pyruvate transamination, and liver and muscle bioenergetics (Babij et al., 1983; Eriksson et al., 1985). This process assists the transport of glucogenic carbons back to working muscle, while also serving as a nitrogen reservoir and buffer for elevated ammonia (Jungas et al., 1992). During exercise, the transamination of pyruvate into alanine in muscle via SGPT (i.e. alanine aminotransferase) elevates alanine in proportion to exercise intensity, duration, and BCAA catabolism (equation (1)) as follows:

$$\underset{\text{(liver)}}{\text{Pyruvate} + \text{Glutamate}} \xleftrightarrow{\text{SGPT}} \underset{\text{(muscle)}}{\text{Alanine} + \alpha\text{-ketoglutarate}} \qquad (1)$$

Alanine is the primary carrier of nitrogen and carbon atoms to the liver for the bioenergetic transfer to pyruvate. In the liver, alanine is transaminated (via SGPT) back to pyruvate (glutamate serving as the nitrogen carrier), which may be transported back to the working muscle for energy.

AA catabolism for energy production yields ammonia as a direct product, and therefore, contributes to both exercise hyperammonemia and OAA depletion (by draining the precursor α-ketoglutarate). This is important, because catabolism of glucogenic AA's characteristically provide the carbon backbone for pyruvate or TCA cycle intermediates (α-ketoglutarate, succinyl-CoA, fumarate, OAA), while ketogenic AA's provide bioenergetic carbons for acetyl CoA (or derivatives). In situations of excessive ammonia production,

such as during high-intensity exercise and/or elevated AA catabolism, draining of the TCA intermediate α-ketoglutarate occurs in the process of glutamate (α-ketoglutarate + $NH_3$ <=> glutamate) and glutamine (glutamate + $NH_3$ <=> glutamine) production (Goldstein, 1976; Levin et al., 1969; Tannen, 1978).

Thus, hyperammonemia and α-ketoglutarate availability limit the desirable production of OAA by the TCA cycle. Further, Ohira et al. (1987) demonstrated that aspartate aminotransferase activity increases with exercise. Therefore, aspartate aminotransferase activity assists OAA homeostasis for maintenance of TCA cycle continuity and efficiency. Additionally, concerning training status, Miller et al. (1987) demonstrated lower aspartate levels with exhaustive exercise and lack of training, indicating regulation of performance by aspartate availability. Further, Lancha et al. (1994) demonstrated with exhaustive exercise in trained and untrained rats supplemented with aspartate and asparagine, a three-fold greater conversion (vs. placebo) of acetyl-CoA and OAA into citrate and coenzyme-A. In another study, Lancha et al. (1995) demonstrated increased plasma aspartate, glycogen content, FFA's, and prolonged (+42%) swim time to exhaustion with supplementation of aspartate, asparagine, and carnitine. These data suggest that OAA and aspartate metabolism link exercise performance, training, and aspartate supplementation by promoting efficiency of TCA cycle bioenergetics with continuity of glycolysis, β-oxidation, and gluconeogenesis.

### 1.2.9 Ammonia Removal: Glutamine, Alanine, Urea Cycle, Pyrimidines

Minor fluctuations in blood ammonia concentration are a normal response to digestion, mild-moderate muscular contraction, and/or elevated AA catabolism (e.g. BCAA's). However, as blood ammonia rises to levels observed in clinical hyperammonemia and intense exercise (> [100 μM/L]), the risk increases for impaired 1) clinical status, 2) metabolic function, and 3) exercise performance. It is therefore desirable

to reduce excess blood ammonia as quickly and efficiently as possible. The following sections summarize the major biochemical mechanisms for the removal of blood ammonia for homeostatic maintenance.

The physiological and nutritional state of the individual affects the flow of carbons (for ATP production) and nitrogens, and the subsequent clearance of waste products such as ammonia. The amino acid(s) glutamine and alanine are the major metabolic depots of ammonia in muscle and blood during exercise (Nurjhan et al., 1995). Both assist the reduction of hyperammonemia, by transporting nitrogen and ammonia in a less-toxic form between the working muscle and liver. While alanine is the major transport form of glucose to and from muscle and the liver, glutamine is the major AA carrier of nitrogen out of muscle, in response to elevated intramuscular ATP and AA metabolism. An increase in blood glutamine concentration and changes in pH promote a shift in glutamine synthetase to produce ammonia in vitro (i.e. glutamine => glutamate + $NH_3$). According to Tannen (1978), the amide groups of glutamine account for 30 - 40% of the $NH_3$ excreted in the urine.

The shifting of the reversible glutamine synthetase (glutamate + $NH_3$<=> glutamine) to the right indicates excessive ammonia in the form of muscle, plasma, or blood glutamine (Babij et al., 1983; Duda and Handler, 1957; Eriksson et al., 1985). During exercise, as ammonia levels rise and arterial glutamate declines, working leg muscles exhibit a significant glutamate uptake -- promoting a rise in muscle and blood glutamine concentration (Babij et al. 1983; Dudley et al., 1983; Eriksson et al., 1985). In addition, the transamination of α-ketoglutarate to glutamate (α-ketoglutarate + $NH_3$ <=> glutamate) supplies additional glutamate substrate for glutamine formation. Therefore, hyperammonemia and the associated glutamate and glutamine sinks drain the TCA intermediate α-ketoglutarate of carbons, and as suggested by data from Lancha et al.

(1994), suggests reduced aspartate and OAA availability with exhaustive exercise, thus inhibiting TCA cycle continuity and athletic endurance.

The primary metabolic pathway for the detoxification of blood ammonia is urea production in the liver by the Krebs-Henseleit urea cycle (Krebs and Henseleit, 1932; Onstad and Zieve, 1979). Urea cycle activity is promoted by elevated concentrations of blood ammonia. The purpose of the urea cycle is to convert (the more toxic) ammonia into (the less toxic) urea for dilution and excretion by the kidneys. The urea cycle becomes more active during exercise, to clear elevated blood ammonia into urea nitrogen to preserve metabolic function. Ammonia enters the urea cycle by reacting with $HCO_3^-$ and two ATP to form carbamoyl phosphate (Katunuma et al., 1966). Carbamoyl phosphate donates the $NH_3$ component as inorganic nitrogen to the urea cycle, while aspartate donates the amino nitrogen while releasing the carbon chain as fumarate. Carbamoyl phosphate then reacts with ornithine to form citrulline (Grossi et al., 1967). Citruilline reacts with incoming aspartate (which donates a nitrogen group, and carries the carbons for fumarate) to form argininosuccinate, producing arginine and fumarate (Briggs and Freedland, 1976). Arginine is cleaved into ornithine and urea, while fumarate is utilized as a substrate in the TCA cycle. Ornithine may combine again with carbamoyl phosphate from ammonia entry, while the less toxic urea (versus that of ammonia) is transported to the kidneys for dilution and excretion in the urine.

Urea synthesis is controlled by the concentration(s) of urea cycle intermediates, and activity of the enzymes carbamoyl phosphatase, argininosuccinate synthetase, and arginase (Buttery and Roswell, 1974; Katunuma et al., 1966; Kramer and Freeland, 1972; Ratner, 1975). Laboratory data indicates that argininosuccinate synthetase has the lowest activity of the urea cycle enzymes (Briggs and Freedland, 1976; Brown et al., 1959; Freedland and Sodikoff, 1962). Together with citrulline accumulation and low concentrations of

succeeding intermediates (argininosuccinate and arginine), these data suggest a limiting rate of argininosuccinate synthetase activity under conditions of hyperammonemia (Brown et al., 1959; Buttery and Roswell, 1974; Ratner, 1975).

At rest, protein intake and urea cycle activity are the controlling factors for blood $NH_3$ levels (Levin et al., 1969). However, during intense exercise decreased hepatic blood flow up to 30% - 70% below normal data (Rowell et al., 1964) and enzyme limitation (Cohen, 1981) restrict urea production (Ferreira et al., 1998). Therefore, urea production is reflective of blood ammonia levels, removal capacity (and efficiency) of the urea cycle, and the physiological state and efficiency of the individual. Progressive symptomatic complications of hyperammonemia result, if ammonia remains highly elevated for prolonged periods.

Clinical studies of hyperammonemia (Batshaw, 1984; Maestri et al., 1996) reveal that additional ammonia detoxification pathways assist the urea cycle for conversion of ammonia into less toxic compounds for excretion. For instance, these pathways are recruited with high ammonia levels produced by 1) excess protein intake, and/or 2) insufficient urea cycle conversion of ammonia into urea. In particular, formation of serial glutamine (glutamine synthetase) and urinary pyrimidines (e.g. hippurate, phenylacetylglutamine, and orotic acid) assist the urea cycle to clear ammonia. In response, the urinary pyrimidine products of orotic acid, uridine, and uricil are elevated (Levin et al., 1969). As noted by Visek (1982), 'the quantity of orotic acid in the urine of rats and man is inversely related to the capacity of the organism for urea synthesis'. Further, data from Visek (1979) demonstrate that orotic acid formation by intoxication with ammonium salts is reduced if animals are pretreated with ornithine, citrulline, or arginine. In addition, Milner and Visek (1974) showed that orotate excretion tends to increase as the percentage of dietary arginine decreases. Thus, medical clinicians suspect urea cycle

defects or insufficiency when, in the resting state, excessive amounts of ammonia, glutamine, and pyrimidine metabolites are excreted indicating saturation of the $K_m$ of urea production. On this note, clinical data from Batshaw (1984) and Maestri et al. (1996) have shown drops in mortality due to hyperammonemia with acute administration of lactulose, sodium benzoate, or sodium phenylacetate to enhance pyrimidine production. However, there are no published reports to date concerning lowering of EIH by sodium benzoate or lactulose, while only one paper to date (Eto et al., 1994) has investigated the effects on blood ammonia during exercise following administration of glutamate (with arginine). Results of this study showed blood ammonia was significantly lower, by up to 40%, during steady-state exercise at ~ 75% $VO_{2max}$ following ingestion of 20 grams of a glutamate-arginine salt in healthy young adult males.

### 1.2.10 Summary

The rise in blood ammonia concentration is a normal response to exercise and elevated AA catabolism (e.g. BCAA's). However, it is apparent that as blood ammonia levels rise to that observed in clinical hyperammonemia (> [100 μM/L]), the risk increases for undesirable metabolic function and impaired clinical status. Therefore, it is desirable to lower and/or remove excess ammonia as quickly and efficiently as possible. Ammonia and aspartate link ATP hydrolysis, AA bioenergetics, and urea metabolism, summarized as follows:

1) $NH_3$ is a by-product of ATP hydrolysis, AA catabolism, and urea formation
2) Aspartate is a substrate in the PNC for AMP resynthesis, promoting desirable maintenance of total adenine nucleotides (AMP + ADP + ATP)
3) Aspartate shuttles high-energy equivalents into the inner mitochondria for ATP production via the electron transport chain
4) The PNC and urea cycle both utilize aspartate to produce the anaplerotic TCA intermediate fumarate
5) Aspartate is an amino nitrogen donor and substrate in transamination, the PNC, and the urea cycle.

### 1.3 Blood Ammonia Response to Exercise

In apparently healthy individuals, resting fasting blood ammonia levels are normally < 50 μM/L (Batshaw, 1984). Strenuous physical activity may elevate blood ammonia concentrations 10-fold and higher, both during and following the period of contraction. Values observed during intense exercise are often well above the minimum of [100 μM/L] for the interpretation of clinical hyperammonemia (Bessman and Bradley, 1955; Katz et al., 1986; Rennie et al., 1981). As reviewed recently by Yuan and Chan (2000), the major bioenergetic sources for $NH_3$ during exercise are AMP deaminase, PNC activity, and/or catabolism of branched chain amino acids (BCAA's). Though muscular ammonia concentrations are several-fold higher versus that of blood ammonia during intense exercise, it is unclear whether the difference reflects primarily the accelerated production in muscle, insufficient intramuscular buffering, or impairment in serial clearance (e.g. restricted hepatic conversion to urea).

Table 1.2 lists various factors that affect blood ammonia concentrations in clinical situations and in response to exercise. Blood levels of ammonia and lactic acid follow similar courses during various levels of exercise intensity. Well-documented phenomena, each is produced (more predominantly) in fast-twitch Type IIb skeletal muscle fibers vs. Type I fibers (Banister, 1983; Banister et al., 1985; Meyer and Terjung, 1979). Blood lactate and ammonia are moderately correlated (r = .6 - .82) during high-intensity work (> 70% $VO_{2max}$) (Babij et al., 1983; Buono et al., 1984; Eriksson et al., 1985). Further, Buono et al. (1984) noted a strong correlation (r = .96) between the alinear rise in blood ammonia and the lactate anaerobic threshold. During intense exercise, blood ammonia concentrations rise 4 to 20 fold, usually reaching ~ 100 - 500 μM/L at exhaustion (Banister et al., 1983; Katz et al., 1986; Wilkerson et al., 1977).

Table 1.2. Factors affecting blood ammonia levels.

| Factor | [$NH_3$] change | Reference(s) |
|---|---|---|
| Gender | Male > Female | Derave et al. (1997) |
| Modality | Bike > Treadmill | Bouckaert and Pannier (1995)<br>Wilkerson et al. (1975) |
| Exercise Duration | ↑ | Brouns et al. (1990),<br>Graham et al. (1995a)<br>(Table 1.2 continued) |
| Exercise Intensity | ↑ | Hegeloch et al. (1990) |
| Endurance Training | ↓ (training specific) | Graham et al. (1997) |
| FT vs. ST Fibers | FT > ST | Gerez and Kirsten (1965)<br>DuRuisseau et al., (1956)<br>Winder et al. (1974) |
| High PRO Diet | ↑ | Czarnowski et al. (1995) Jungas et al. (1992) |
| Ambient Heat | ↑ | Snow et al. (1993 |
| Arm training | ↓ (training specific) | Nelson (unpublished data) |
| Leg training | ↓ (training specific) | Nelson (unpublished data) |
| Creatine Loading | ↓ | Greenhaff et al. (1993) |
| β-blockade | ↑ | Jensen-Urstad et al. (1993) |
| Urea Cycle Disease | ↑ | Lockwood et al. (1979) |
| McArdles Disease | ↑ | Wagenmakers et al. (1990) |
| BCAA ingestion | ↑ | Calders et al. (1997) |
| Urea Cycle Substrate Supplementation | No change | Edwards and Day (2000) |
| Urea Cycle Substrate Supplementation | ↓ (clinical) | Greenstein et al. (1956) |
| Urea Cycle Substrate Supplementation | ↓ (clinical) | Grossi et al. (1967) |
| Carnitine Supplementation | ↓ | Costell et al. (1984)<br>Sarhan et al. (1994) |
| Benzoate | ↓ | Kubota and Ishizaki (1993) |
| Aspartate Supplementation | ↓ | Kendrick (1976) |
| Zinc Supplementation | ↓ | Riggio et al. (1992) |

On the other hand, immediately following intense exercise blood ammonia concentrations are more variable versus lactate (Babij et al., 1983; Buono et al., 1984; Meyer and Terjung, 1979; Schwartz et al., 1958). Blood $NH_3$ may rise, stabilize, or decline

rapidly versus the well-known lactate rise following intense exercise (Banister et al., 1983; Schwartz et al., 1958; Wilkerson et al., 1977). $NH_3$ clearance is affected by specificity of training, status of conditioning, terminating intensity, and exercise duration. In general, a more efficient metabolic state (i.e. a higher level of state of training) lowers blood ammonia concentration at submaximal exercise intensities, enhances ammonia reduction during recovery, and enhances performance (Graham et al., 1997). Concerning training status and blood ammonia concentrations, Graham et al. (1995a) and Graham et al. (1997) note that highly-trained subjects exhibit:

1) Lower blood ammonia at a given exercise intensity (vs. untrained),
2) Faster clearance following exercise,
3) Longer endurance at a given ammonia level, and
4) Longer time (vs. untrained) to reach a given ammonia blood level at exhaustion.

**1.4 Physiological Impact of Elevated Ammonia**

Several physiological, metabolic, and nutritional factors mediate blood ammonia levels. For example, the following raise blood ammonia concentration:

1) Increasing exercise intensity (i.e. increasing AMP deaminase, PNC activity) (Hellsten et al., 1999),
2) Male gender (versus females) (Lynch et al., 2000),
3) Glycogen depletion (Broberg and Sahlin, 1988),
4) High protein/low carbohydrate diet (i.e. ammonia produced by gut flora urease, BCAA metabolism) (Greenhaff et al., 1991; MacLean et al., 1992; Snow et al, 2000),
5) Heat (Snow et al., 1993),
6) β-blockade (Jensen-Urstad et al., 1993), and
7) BCAA ingestion (MacLean et al., 1996).

Further, as reviewed by Mutch and Banister, (1983), ammonia is produced endogenously in the brain due to the GABA cycle and the deamination of brain catecholemines, and as suggested by data from Cooper et al. (1979) and Lockwood et al. (1979) $NH_3$ may cross the blood brain barrier. Additional data from Hindfelt (1983), Lockwood et al., (1979), and Najarian et al. (1958) indicate that **e**levated brain ammonia affects energy metabolism and

leads to mental confusion, ataxia, convulsion, and cramps in both animals and man. With prolonged exertion, and/ metabolic and contractile stimuli blood ammonia levels rise, and fatigue results. Fatigue is generally agreed to be due to a variable combination of 1) a depleted substrate supply (i.e. ATP, phosphocreatine, glycogen, glucose), 2) accelerated undesirable metabolite (e.g. lactate) and waste ($CO_2$, $NH_3$, $H^+$) formation, and 3) disruption of central nervous system function (e.g. buildup of tryptophan and seratonin).

Figure 1.1 highlights the deleterious effects of hyperammonemia. The cascading deleterious effects of ammonia on exercise metabolism are largely a result of a lack of training coupled with accelerated sympathetic discharge. For instance, epinephrine, cortisol, and/or hypoxia increase ammonia output, in part, because of the catabolism of the protein/AA content (i.e. muscle proteins, BCAA's, aspartate) of the tissue (Goodman and Lowenstein, 1977) to keep up with ATP demand. The major rate-limiting enzyme of glycolysis, phosphofructokinase (PFK), is inhibited by physiological levels of ATP at acidic pH values (Lowenstein, 1972; Mutch and Banister, 1983), but, is stimulated by [$NH_3$]. Work by Felig and Wahren (1971), Lowenstein (1972), and Sugden and Newsholme (1975) indicate that stimulation of PFK by ammonia occurs without a change in pH, thus increasing the availability of pyruvate for the pyruvate/alanine cycle which further depletes glycogen and glucose.

Ammonia further inhibits liver mitochondrial respiration (and citrate and isocitrate oxidation). Data from Katanuma et al. (1966) and Brylla and Nidzwiecka (1979) indicate that $NH_3$ accumulation inhibits both pyruvate carboxylation and decarboxylation - the initial steps in 1) gluconeogenesis, and 2) the conversion into acetyl Co-A for TCA entry via glycolysis, respectively. Thus, elevated blood ammonia accelerates non-oxidative carbohydrate metabolism for ATP production, as well as inhibiting oxidative ATP production.

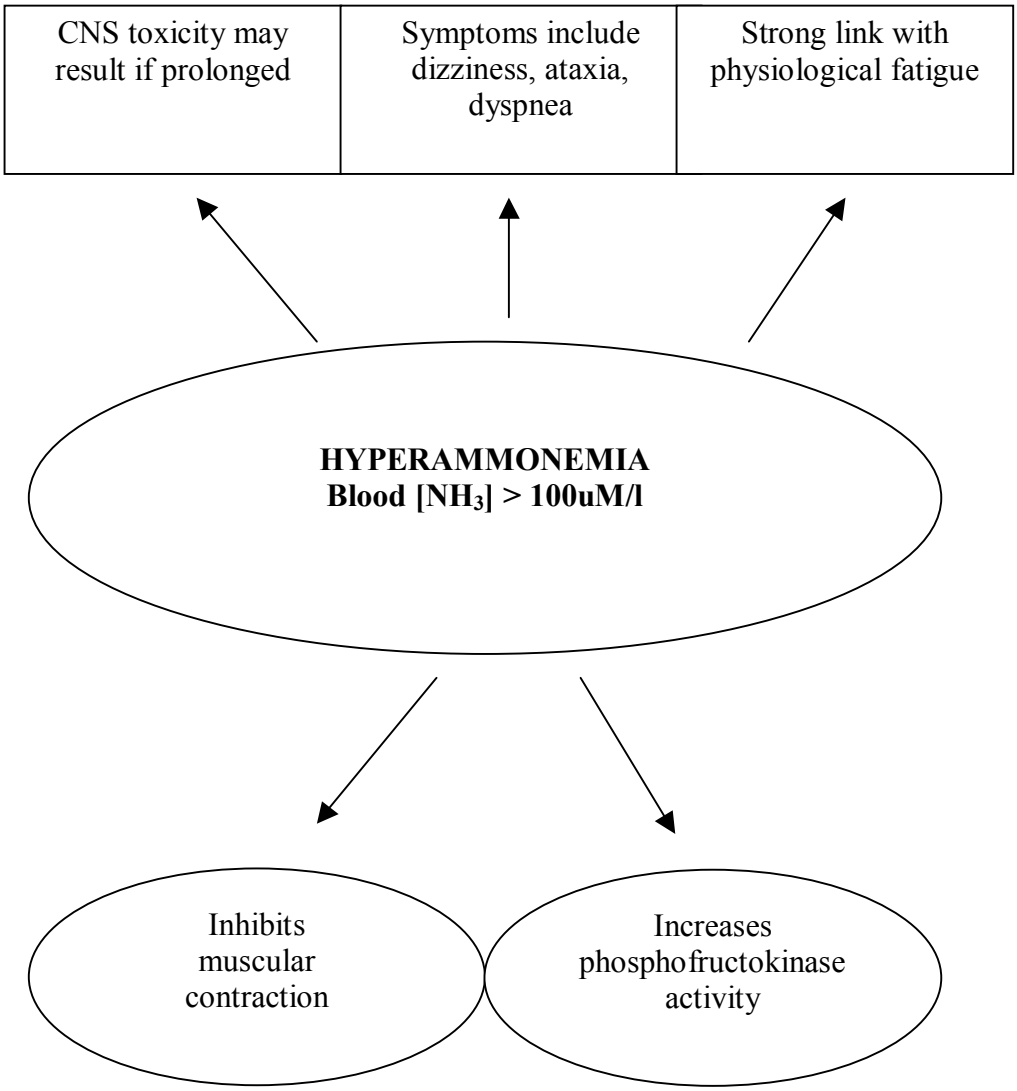


Figure 1.1. Deleterious effects of hyperammonemia.

Ammonia flux thus affects the ATP/ADP ratio, acid base balance, glycolytic activity, pyruvate carboxylation and decarboxylation, and tricarboxylic acid cycle activity (Banister et al., 1985; Hindfelt, 1983; Lowenstein, 1972). Further, ammonia increases $V_E$, and contributes to hyperventilation (Hindfelt, 1983; Mutch and Banister, 1983). Mechanisms include elevated activity of respiratory muscles, and/or direct stimulation/interference of humoral input to the respiratory center (Hindfelt, 1983; Mutch and Banister, 1983). Ammonia is a weak base, and reacts reversibly with $H^+$ to produce ammonium ($NH_3 + H^+ <=> NH4^+$). Exercising muscle retains up to 90% of $NH_3$ within the muscle cell as $NH_4^+$ (Katz et al., 1986; Rosado et al., 1962). The cellular membrane is highly permeable to $NH_3$, but has a poor permeability to $NH_4^+$. Thus, the release of $NH_3$ into the circulation is associated with a net loss of hydrogen ions (Katz et al., 1986). As demonstrated by Heald (1975), it appears desirable to reduce high levels of intramuscular ammonia to maintain electrical excitability and tension of individual muscle fibers.

Historically, as reviewed by Lantigua et al. (1980) and Felig (1984), concerns have arisen over the adverse effects (e.g. 'electrocardiographical abnormalities', 'serious arrhythmias', and 'cardiac disturbances') in 3 of the 6 cases precipitating death, from accompanying nutritional hyperammonemia in those on high liquid protein diets. Despite the high rate of success for achieving weight reduction, results from these cases indicate that those on very low calorie (< 500 kcal per day) protein diets utilizing a base protein with very low biological value (e.g. collagen hydrosylate) are at an even higher risk for adverse consequences. According to Felig (1984), in the 1970's when liquid-protein diets were the rage for the 'over the counter' treatment of obesity, 60 deaths were reported in over 100,000 participants using this protein as the primary source of nutrition. Further

inspection by Isner et al. (1979) found that in (at least) 17 of the cases, use of the liquid protein (with low biological value) diet for two months was implicated as the basis for the ventricular arrhythmias that precipitated death. Further, in some of these cases, extended hyperammonemia was considered a precipitating factor exacerbating adverse metabolic effects. According to Jackson (1999), the most toxic of the individual amino acids is methionine; and, the adverse effects of methionine are more evident on a low protein diet than with adequate protein intake. Furthermore, data from Riggio et al. (1992) suggests a link between zinc deficiency and impairment of the urea cycle enzyme ornithine transcarbamylase. Thus, the lack of dietary zinc inherent in a low protein diet reduces the efficiency of ammonia removal. Therefore, the potential for hyperammonemia (and associated adverse effects) increases with elevated protein intake, and especially in those on low calorie diets using a higher percentage of protein. In these situations, it is apparent that the use of a protein with high biological value is of greater importance, as is consideration of supplementation with minerals and ammonia reducing amino acids (e.g. aspartate, arginine, ornithine, citrulline).

## 1.5 Impact of Reducing Hyperammonemia: Clinical Trials and Exercise Trials

### 1.5.1 Clinical Trials

Historically, treatment for reducing clinical hyperammonemia (Batshaw, 1984; Najarian et al., 1958) targets:

1) Inhibition of bacterial urease (i.e. neomyocin),
2) Supplementation with ammonia reducing amino acid(s) in the pathways of transamination (glutamate therapy, i.e. $NH_3$ + glutamate <=> glutamine) and urea synthesis (e.g. aspartate, arginine, and ornithine), and
3) Administration of agents (e.g. sodium benzoate, phenylacetate, lactulose) to enhance pyrimidine synthesis (e.g. orotic acid).

Current clinical methods to lower hyperammonemia (i.e. in cases of hepatic encephalopathy) include 1) restricting dietary protein intake, 2) enhancing the

detoxification reactions in the body (transamination, urea cycle, pyrimidine production) (Levin et al., 1969; Mutch and Banister, 1983), and 3) correcting the metabolic etiology. In humans, administration of up to 25 grams of arginine, ornithine, glutamate, and/or benzoate reduces clinical hyperammonemia (CH) and associated symptoms (Artz et al., 1958; Najarian et al., 1958; Strauven et al., 1976). Clinical settings currently utilize ammonia-reducing amino acid(s) (e.g. aspartate, ornithine, glutamate, and arginine) in combination with metabolic agents (i.e. sodium benzoate, carnitine, and lactulose) to significantly and consistently reduce CH and hepatic encephalopathy and improve prognosis. The following paragraphs discuss the rationale and clinical impact of reducing hyperammonemia and treating fatigue with supplementation of ammonia reducing (and/or urea producing) amino acids.

The addition of the urea cycle intermediate(s) arginine or ornithine in vitro accelerates urea formation in liver slices (Krebs and Henseleit, 1932; Kubota and Ishizaki, 1993). In clinical trials, Fahey (1957) and Fahey et al. (1958) further demonstrate that administration of arginine (or other urea cycle intermediates such as ornithine, citrulline, aspartate) eliminates $NH_3$ accumulation and the associated toxic effects (death, convulsions). Data in laboratory animals from Winitz et al. (1956) and Salvatore et al. (1964) show that elevations in blood ammonia and urea accompany a lethal dose of ammonium acetate, and, the toxic effects are removed once arginine, ornithine, and/or aspartate are administered. Fahey (1957) and Fahey et al. (1958) demonstrated in humans that administering an essential amino acid (AA) infusion that lacked arginine and histidine exacerbated CH in patients without 'any evidence of liver disease'. Further, the associated symptoms of hyperammonemia – (e.g. nausea and retching) were reduced or eliminated when L-Arginine, L-Ornithine, or L-Citrulline were added at a molar concentration of 1-

2%. L-Ornithine appeared to be equally as effective as L-Arginine, L-Citrulline slightly less effective, while D-Arginine and L-Glutamate had no protective effect.

These cases illustrate that the severity of clinical hyperammonemia and associated symptoms are reduced when the L-amino acids in the urea cycle are supplemented in an AA infusion. As shown by Fahey et al. (1958), when no urea cycle substrate is supplemented in an AA infusion, AA's peak immediately after infusion, blood ammonia peaks 2 hours after, and plasma urea peaks 4 hours after. However, when the infusion includes urea cycle L-AA's (such as L-Arginine), 1) the alpha amino changes are not altered, 2) urea peaks earlier, and 3) ammonia does not rise at all (Fahey et al., 1958). Further, the administration of amino acids whose metabolism is outside of the urea cycle (e.g. BCAA's) exhibit little or no effect toward the reduction of ammonia, and in fact, some AA's raise $NH_3$ levels and exacerbate associated symptoms further (Fahey, 1957; and Fahey et al., 1958).

Clinical studies in the 1950's and the early 1960's revealed improvements in clinical prognosis of hyperammonemia following administration of aspartate and/or urea cycle intermediates. Shortly thereafter, aspartate supplementation was found effective for the clinical treatment of fatigue (New drugs and Spartase, 1963). Prescribed initially in a dosage of 1g x BID for the management of fatigue syndrome (New drugs and Spartase, 1963), the theoretical basis for aspartate treatment is to provide additional substrate to the urea cycle, enhancing the clearance of the more toxic metabolic ammonia into the less toxic urea, and thus improve clinical outlook. The following series of papers trace the development of aspartate supplementation for the treatment of clinical fatigue.

In a 4-week double-blind study by Shaw et al. (1962), 57 patients complaining of fatigue without demonstrable organic disease received potassium and magnesium aspartates, while 28 similar patients received a placebo. By the end of the study, 49 of the

57 patients (86%) receiving aspartates noted improvement both during the day and after a full day's schedule, compared to 7 of 28 (25%) with placebo. Response in the experimental group generally took about 4 days to take effect.

Fornica (1962) conducted a placebo-controlled study involving 84 women and 16 men with persistent tiredness believed to be unrelated to depression. Some of the patients had been symptomatic for more than two years. Aspartate was administered to 6% of the group for 2 weeks, and to 68% for 4-6 weeks. The remaining 26% received placebo. A positive therapeutic response developed gradually over 4 - 10 days in 87% of the aspartate treated patients. After 4-6 weeks, most improved patients stopped the supplements and continued to do well.

Hicks et al. (1964) conducted a double-blind study of 145 patients, whose fatigue had a variety of bases, including anxiety neuroses, gastrointestinal disturbances, menopause, the postpartum period and fatigue as a sequel to influenza. Of those, 85% of those given potassium and magnesium aspartates reported an increase in strength or physical activity compared to only 9 % of patients on the placebo.

In conclusion, as reviewed by Gaby (1982), clinical trials have shown that in nearly 3,000 patients, 75-91% experienced pronounced relief of fatigue with supplements of potassium and magnesium aspartate. In general, one gram of aspartate daily improves prognosis from clinical fatigue within 4 - 10 days of supplementation. Therefore, results from these studies suggest that 1 gram QD of aspartate is generally effective in the treatment of clinical fatigue.

### 1.5.2 Exercise Trials: Introduction

It is well documented that physical activity elevates blood ammonia (Parnas, 1929; Wilkerson et al., 1975; and Edwards and Day, 2000;). Exercise-induced hyperammonemia (EIH) is detrimental to short-term maintenance of optimal high-intensity physical function

and performance (Heald, 1975; Washio and Mashima, 1963). Historically, clinical therapy for hyperammonemia has utilized administration of urea cycle intermediates (i.e. arginine, ornithine, aspartate), glutamate, and conjugated conversion (e.g. via administration of sodium benzoate, lactulose) of ammonia into urinary pyrimidines (Kubota and Ishizaki, 1993). Despite clinical success for reducing hyperammonemia by the intake of ammonia-reducing amino acids in the urea cycle (e.g. arginine, ornithine, aspartate, citrulline), only a handful of investigations report the possible performance enhancement of these in healthy populations. Exercise studies show little or no effect (Denis et al., 1991; Eto et al., 1994; and Edwards and Day, 2000) for reducing EIH, and no effect on performance enhancement, with supplementation of ammonia-reducing amino acids in the urea cycle.

On the other hand, animal studies report success with aspartate supplementation to improve exercise performance; however, reproduction of results is inconsistent. The most popular theory for performance enhancement is that aspartate supplementation reduces blood ammonia accumulation during exercise (Washio and Mahima, 1963; Heald et al. 1975; and Mutch and Banister, 1982). However, as presented in Table 1.3, the anaplerotic roles of aspartate may provide clues for the mechanisms of how aspartate supplementation improves exercise performance. These include 1) contributing carbons to the TCA cycle (i.e. OAA, fumarate), and/or 2) contributing metabolic substrate (aspartate) for the PNC (i.e. AMP maintenance) and transamination (i.e. carbon and nitrogen transfer).

Overall, methodologies, biochemical mechanisms, and target groups for aspartate supplementation for performance improvement are not established. Animal studies are presented in Table 1.4, and are discussed in Section 1.5.3; human studies are presented in Table 1.5, and discussed in Section 1.5.4. An overall summary and conclusions from the animal and human studies is presented in Section 1.6.

Table 1.3. Anaplerotic roles of aspartate.

| Metabolic Pathway(s) | Aspartate Role | Key Enzyme(s) | Bioenergetic Result |
|---|---|---|---|
| Oxidative deamination | Carbons for OAA | GDH | ↑ [OAA] |
| Transamination | Transfer of amino group to glutamate | SGOT | ↑ sparing of α-ketoglutarate |
| Urea Cycle | Substrate | Argininosuccinate synthetase | ↓ [$NH_3$]<br>↑ [Fumarate] |
| Malate/Aspartate Shuttle | Shuttle NADH across inner mitochondrial membrane in the ETC | NADH dehydrogenase | ↑ ETC efficiency<br>↑ [ATP] |
| Purine nucleotide cycle | Substrate | Adenylosuccinate synthetase<br>AMP deaminase | TAN maintenance<br>↑ [Fumarate]<br>AMP regeneration |

SGOT – serum glutamate oxaloacetate transaminase; OAA – oxaloacetate
GDH – glutamate dehydrogenase; NADH – nicotinamide adenine dinucleotide
ETC – electron transport chain; ATP – adenosine triphosphate;
AMP – adenosine monophosphate

### 1.5.3 Exercise Trials: Animal Models

Table 1.4 summarizes the studies of aspartic acid supplementation to improve performance in animals. The first reported study of aspartic acid supplementation for exercise performance in either animals or man was conducted by Laborit et al. (1958). In this study, administration of aspartic acid prolonged swim time to exhaustion in rats while lowering blood $NH_3$, which indicated that exhaustion was related to the level of blood $NH_3$.

Additional animal studies have shown blood ammonia reduction and performance enhancement with dosages up to 500 mg/kg (Barnes et al., 1964; Kendrick, 1976; Glass et al., 1994). However, above this amount, the beneficial effect (i.e. reducing

Table 1.4. Summary of studies of L-Aspartic Acid and performance in animals.

| Group | Protocol | Dosage (daily) | Performance | Reference |
|---|---|---|---|---|
| Rats | Swim to exhaustion | 50 mg/kg | ↑ | Laborit et al. (1958) |
| Rats | Swim to exhaustion | 1000 mg/kg | ↑ | Rosen et al. (1962) |
| Rats | Swim to exhaustion | 500 mg/kg | ↑ | Barnes et al. (1964) |
| Rats/ dogs | Swim to exhaustion | 500-2000 mg/kg | no change | Matoush et al. (1964) |
| Rats | Swim to exhaustion | 12.5 mg/kg | ↑ | Kendrick (1976) |
| Rats | Swim to exhaustion | 12.5 mg/kg | ↑ | Tangsakul (1977) |
| | | 125 mg/kg | ↑ | |
| | | 250 mg/kg | no change | |
| | | 1000 mg/kg | ↓ | |
| Rats | Swim to exhaustion | 1000 mg/kg | no change | Trudeau and Murphy (1993) |
| Rats | Swim to exhaustion | 6.25 mg/kg | ↑ | Glass et al. (1994) |
| | | 12.5 mg/kg | ↑ | |
| | | 100 mg/kg | ↑ | |
| Rats | Swim to exhaustion | 45 mg/kg each aspartate, asparagine, carnitine | ↑ | Lancha et al. (1995) |
| Rats | Swim to exhaustion | 200 mg/kg | ↑ | Nichols et al. (1998) |
| Rats | Swim to exhaustion | 500 mg/kg | ↑ | Winters et al. (1998) |

hyperammonemia and/or enhancing performance) appears to taper off, and then have no benefit at all, with dosages equaling or exceeding 1000 mg/kg (Matoush et al., 1964; Tangsakul, 1977; and Trudeau et al., 1993). Results from the animal studies are discussed in the next paragraph in chronological order.

Rosen et al. (1962) investigated the effects of aspartate administration on two groups of 40 untrained male rats swimming to exhaustion in 29 degrees C water. Results showed that 1000 mg/kg aspartate administration increased time to exhaustion by 15% in the aspartate group (195 minutes, placebo vs. 224 minutes, aspartate). Blood ammonia concentrations were not reported. Results from this study suggest that 1000 mg/kg aspartate supplementation improves long-duration time to exhaustion in swimming rats

Barnes et al. (1964) investigated the effects of aspartate administration on blood ammonia and time to exhaustion in a series of three studies. One hundred and twelve male (trained and untrained) rats were utilized in a swimming protocol in groups of n = 4 - 10 each. In studies 1 and 2, the supplementation was a twice-administered dose of 500 mg/kg, while study 3 utilized a single dose, 500 mg/kg. Water temperature was 25 degrees C for all experiments. In study 1 (untrained rats), time to exhaustion increased from ~16 minutes to ~ 23 minutes with aspartate administration vs. placebo. Blood ammonia concentrations were lower with aspartate treatment, (61 vs. 113 [μM/L]). In study 2, investigating the effects of prior training, time to exhaustion again responded favorably with supplementation, from 20 min (placebo) to 25 min (aspartate). Blood ammonia concentrations in the trained group (3 weeks training) were also lower with aspartate treatment, 48 μM/L (placebo) vs. 42 μM/L (aspartate). In study three, with one dose of aspartate (500 mg/kg) in untrained rats, blood ammonia levels after a short period (~ 10 minutes) of swimming were lower, [49 μM/L] versus [103 μM/L], respectively. These results indicate that both trained and untrained rats responded favorably (in both exercise

performance and blood ammonia clearance) with short-term supplementation of 500 mg/kg aspartate.

Matoush et al. (1964) investigated the effects of ~ 2000 mg/kg aspartate supplementation on swim time to exhaustion in 40 rats (trained and untrained) and four dogs in 17 degrees C and 25 degrees C water. There were no significant differences in any of the groups, trained or untrained, in response to aspartate treatment. Although no blood ammonia values were reported, one may speculate from this and other studies (Tangsakul, 1977; and Trudeau et al., 1993) that a dosage of 2000 mg/kg may be too high to elicit a positive effect on exercise-induced hyperammonemia, or have a beneficial effect on performance, regardless of training status.

Cutinelli et al. (1970) investigated the effects of 200 mg/kg ornithine-aspartate (versus 0.9 % saline) administered 30' prior to swimming 10 to 60 predetermined minutes in 165 male untrained rats. Blood ammonia concentrations were higher than at 20 degrees C, than at 28 degrees C, with no significant difference between treatments. Further, in the 10 minute swim test at 20 $C^{o}$, supplementation with ornithine-aspartate (versus placebo) decreased ATP, decreased creatine, and decreased GPT, with no difference in AMP and ADP. However, versus a control group who received no treatment or exercise, these values were all significantly higher in the supplemented group. Thus, these results suggest that ornithine-aspartate supplementation can enhance ammonia clearance and maintain AMP and ADP nucleotides in response to high-intensity exercise.

In two unpublished doctoral dissertation(s), Tangsakul (1977) and Kendrick (1976) demonstrated improved exercise tolerance in rats treated with chronic and acute aspartate administration. In support of the findings of Matoush et al. (1964), animals treated over a 10-day period with a large aspartate dose (~ 4g/kg/day) exhibited no change (or a decrement in) in WTE. Further, an acute injection of aspartate, in smaller dosage(s) one-

hour before exercise, demonstrated the following dose-dependent responses in work time to exhaustion (WTE) (Kendrick, 1976):

1) 1g/kg reduced WTE by ~ 41% below that of control
2) 250 mg/kg did not alter WTE;
3) 125 mg/kg increased WTE by ~37%, and
4) 12.5 mg/kg K-aspartate increased WTE 90%.

These data indicate that L-Aspartic Acid intake affects WTE in a dose-dependent fashion, and secondly, as in the Matoush et al. (1964) study, dosages exceeding 500 mg/kg intake may inhibit and deter metabolism and performance, respectively. Results have found that rats pretreated with aspartate, buffer the hyperammonemia brought by both exercise and ammonium injection (Barnes et al., 1964; Kendrick, 1976). Additional results from Kendrick (1976) indicate that aspartate administration may not alter the rise in the muscle ammonia concentration during exercise to the degree that blood ammonia is lowered. The authors speculated that the reduction in [blood ammonia] versus [muscular ammonia] was likely due to a greater uptake of ammonia by the liver for urea synthesis. This contention was supported when the administration of K-aspartate significantly increased blood urea during work, with no effect on muscle ammonia (Kendrick, 1976). Therefore, these results suggest the acute reduction of excessive [blood ammonia] and accompanying symptoms (e.g. inhibition of muscular contraction, muscular dysfunction, and central fatigue) are associated with an increase in exercise performance. Further, this study, like several others, showed that improvements in swim time to exhaustion in rats may occur with aspartate supplementation as low as 12.5 mg/kg (Glass et al., 1994) versus the ~100 - 1000 mg/kg observed in previous experiments.

Trudeau and Murphy (1993) investigated the effects of 1000 mg/kg aspartate on swimming performance in a 58 rats in a six-group double blind study investigation. Time to exhaustion was not significantly different with aspartate supplementation (174 ± 45 min)

versus saline treatment (179 ± 38 min). Plasma [FFA] were significantly lower ($p \leq 0.0001$) with aspartate supplementation (0.7 ± 0.3 mM vs. 1.2 ± 0.5 mM). There were no significant differences between treatments for plasma ammonia, glucose, lactate, or muscle (or liver) glycogen. Results from this study fail to support the prior contentions of Ahlborg et al. (1968), and Wesson et al. (1988), that the performance benefit of aspartate supplementation is due to a general glycogen sparing. It is possible, that although lower plasma [FFA] were found in this study, a dose of 1000 mg/kg may be too high (versus the ~ 70 – 150 mg/kg in Ahlborg et al., (1968), and Wesson et al. (1988)) to elicit beneficial effects in other hypothesized markers (blood ammonia concentration, muscle and liver glycogen) and long duration exercise performance.

A series of three abstracts by Drexel University reported the effects of various aspartate dosages on the exercise performance of rats. Glass et al. (1994) examined the effect of 0, 6.25, 12.5, or 100 mg/kg aspartate on swim time to exhaustion in 24 female rats. Aspartate supplementation was associated with a 'dose-related increase in swim time (< 2-fold) in all rats'. Water temperature was a constant 30 degrees C. Nichols et al. (1998) investigated the effects of 200 mg/kg of aspartate supplementation, and/or carbohydrate loading, on swim time to exhaustion in untrained rats. Carbohydrate loading resulted in a 50% increase, aspartate loading resulted in a 3-fold increase, while the combination of the two resulted in a 3.4-fold increase. Winters et al. (1998) investigated the effects of 500 mg/kg of aspartate supplementation on swim time to exhaustion in female untrained rats, one hour after oral gavage. The aspartate treatment resulted in a two-fold increase in time to exhaustion versus water intake only. Water swim temperature was 30 degrees C. These results are in general agreement with the previous investigations of aspartate supplementation $\leq$ 500 mg/kg in rat studies (Laborit et al., 1958; Barnes et al., 1964; and Nichols et al., 1998) to improve swimming performance.

Therefore, the regime of aspartate supplementation to improve exercise tolerance in rats (trained and untrained) appears to be 12.5 mg/kg - 500 mg/kg BID, over the 24 hours preceding exhaustive exercise. Of note is that: 1) most of the animal studies utilized swimming methodology, and 2) improvements in performance and reductions in ammonemia are independent of both training status and gender. A possible explanation for the performance benefit observed in these studies is that aspartate supplementation may delay the onset of an ammonia threshold by lowering exercise ammonia (similar to the lowering of the lactate threshold by training), and thus prolong high-intensity endurance and performance. Further, serial data from Wesson et al., (1988), Trudeau and Murphy (1993), and Lancha et al. (1995) demonstrate that aspartate supplementation reduces plasma [FFA] in response to intense medium-to-long duration exercise. The mechanism and the impact of this are unclear. However, Trudeau and Murphy (1993) found no differences in muscle or liver glycogen (between aspartate supplementation and placebo) at rest or exhaustion, to support a glycogen sparing effect. Two other possibilities exist for the lowering of [FFA] by aspartate supplementation: one, as cited by Brooks et al. (1996), is reduced epinephrine activity on adipose tissue which reduces lipase; two, aspartate supplementation may provide substrate for TCA cycle intermediates and the malate aspartate shuttle, enhancing transport of reducing equivalents across the inner mitochondrial membrane for ATP production. As evidenced recently (Gibala et al., 1997) in short-term (< 5 min) activity, large increases in succinate, malate, and fumarate (> 90% of the total TCA cycle substrate pool) are observed during the first minute of muscular contraction. Therefore, TCA cycle activity is accelerated during short-term intense exercise. Further, additional aspartate substrate in these circumstances may increase production of fumarate by the PNC and urea cycle. Additional work is needed to provide further insight into the etiology of the lowering of FFA and improvement of performance

with aspartate supplementation. In conclusion, results from the animal studies suggest that supplementation with ~ 12.5 – 200 mg//kg of aspartate over 24 hours improves swimming performance in rats, independent of gender and training status.

### 1.5.4 Exercise Trials: Human Models

Table 1.5 summarizes the studies of aspartic acid supplementation to improve performance in humans. In comparison to the animal studies, the results from human studies of aspartate supplementation are more widely variable. In contrast to the animal studies which use primarily swimming as the testing modality, the human studies have used cycling (e.g. Maughan and Sadler, 1983; and Wesson et al., 1988), treadmill (Hagan et al., 1982), weight lifting (Fallis et al., 1963; and Tuttle et al., 1995), field training (Consolazio et al., 1964), outdoor running (Ritter et al., 1998; and Colombani et al., 2000), and static arm force (DeHaan et al., 1985). Though inconclusive, the most significant reductions in EIH and improvements in exercise performance with aspartate supplementation occur with 1) subjects that are highly-trained to the modality, 2) short term high-intensity exercise with large muscle groups, and 3) a protocol that produces a sufficient amount of ammonia to be classified as exercise-induced hyperammonemia. The first reported study of aspartic acid supplementation for enhancement of exercise performance in humans was by Fallis et al. (1963). In general, aspartate dosages have ranged from 1 - 15g QD, with the most significant findings for improving performance occurring with dosages between 5 - 15g (e.g. Ahlborg et al., 1968; Wesson et al., 1988), although significant improvements have occurred with smaller dosages (Gupta and Srivastava, 1973; and Franz and Chintanaseri, 1977). Critique of the human studies is presented in the following paragraphs in chronological order.

Fallis et al. (1963) published a study that investigated the effects of 1g of daily aspartate supplementation versus placebo on weight lifting performance. Twenty-six

Table 1.5. Summary of studies of L-Aspartic Acid and performance in humans.

| Protocol | Dosage (daily) | Performance | Reference |
|---|---|---|---|
| Weight lifting | 1000 mg | no change | Fallis et al. (1963) |
| Field training | 2000 mg | no change | Consolazio et al. (1964) |
| ~80% $VO_{2max}$ to exhaustion (cycling) | 7000 mg | ↑ | Ahlborg et al. (1968) |
| 60% $VO_{2max}$ to exhaustion (cycling) | 3500 mg | ↑ | Gupta and Srivastava (1973) |
| 73.5% W170/kg to exhaustion | 2200 mg | ↑ | Franz and Chintanaseri(1977) |
| 62% $VO_{2max}$ to exhaustion (treadmill walking) | 7200 mg | no change | Hagan et al. (1982) |
| 75% $VO_{2max}$ to exhaustion (cycling) | 6000 mg | no change | Maughan and Sadler (1983) |
| 40% max static arm force | 7640 mg | no change | De Haan et al. (1985) |
| 75% $VO_{2max}$ to exhaustion(cycling) | 10,000 mg | ↑ | Wesson et al. (1988) |
| Weight lifting | 150 mg/kg | no change | Tuttle et al. (1995) |
| One mile run time | 1440 mg | ↑ | Ritter et al. (1998) |
| Marathon run | 15,000 mg | no change | Colombani et al. (2000) |

inmates of the Maryland State Penitentiary volunteered for the study. Subjects were experienced weight lifters, considered to be at peak performance. In a counterbalanced order, the subjects were given placebo for 10 days, and aspartate for 10 days, and recorded the maximum daily number of bench presses, squats, and recovery time. No significant differences were found between treatments. Blood ammonia concentrations were not reported in this study. However, exercise protocols of this type (i.e. weight training) in

general do not raise blood ammonia to the levels of hyperammonemia for a sustained period for aspartate to have an effect.

Consolazio et al. (1964) investigated the effects of 2 grams of daily supplementation of aspartic acid on the respiratory responses to 5 weeks of aspartate intake. Twelve healthy young men were matched for body weight, and randomly assigned to an aspartate group or a placebo group. A 9-week study, with two weeks of initial training, revealed no significant differences between groups in $VO_2$, $VCO_2$, $O_2$ debt, RQ, recovery after an all-out run, maximal breathing capacity, or timed vital capacity. The authors concluded that there was no convincing evidence of the beneficial effects of aspartate therapy for delaying the onset of physiological fatigue in humans, in terms of respiratory parameters. Unfortunately, neither blood ammonia nor blood urea values reported, nor was there a high-intensity exhaustive component to assess effectiveness of treatment. Thus, aspartate supplementation coupled with a training regime has no effect on improvement in respiratory parameters.

Ahlborg et al. (1968) conducted a controlled study on six moderately-trained men, who exercised to exhaustion on a bicycle ergometer for 4 consecutive days at a workload designed to elicit exhaustion at ~ 90 minutes. Placebo was administered on days 1, 2, and 4, and 7 grams of aspartate was administered on day 3. Mean work times to exhaustion were not significantly different between days 1, 2, and 4; however, on day 3, subjects cycled for ~ 128 min. - an increase in endurance by ~ 50%. There were no significant differences in heart rate, respiratory rate, or weight change. It was hypothesized by the authors, that the administration of aspartate decreased the rate of glycogen utilization and therefore prolonged exercise time. However, the study did not utilize a double blind design, nor report blood ammonia or urea to substantiate these conclusions.

A similar follow-up study on five subjects ($VO_{2max}$ - 42.5 ml/kg/min) by Gupta and Srivastava (1973) demonstrated an increase in exercise work time to exhaustion with aspartate intake. Five healthy young volunteers with a baseline $VO_{2max}$ of 42.5 ml/kg/min exercised at a continuous workload of 60% $VO_{2max}$ on a bicycle ergometer, with a 22.6% improvement (68.4 min vs. 83.6 min) in WTE following 3.5 g (.875 g x 4) of aspartate supplementation versus placebo. $VO_2$, HR, $O_2$ debt, urinary creatine, blood sugar, and urinary 17-hydroxycorticosteroids were not significantly different between trials. The subjects in both the Ahlborg et al. (1968) and Gupta and Srivastava (1973) studies were allowed intake of cold water ad libitum during exercise to avoid dehydration. Blood ammonia and urea concentrations were not reported, nor was the single or double-blind nature specified. Therefore, other than no change in blood glucose, the possible mechanisms for improvement in this study are difficult to ascertain.

Hagan et al. (1982) investigated physiological responses to treadmill walking for 90 minutes at approximately 62% $VO_{2max}$ in response to $K^{+}Mg^{+2}$ aspartate treatment (7.2g over 24 hours), placebo, or no treatment in seven healthy males ($VO_{2max}$ 59.5 ml/kg/min). No significant differences were observed in $VO_2$, $VCO_2$, R, HR, and BP; and for pre- and post-exercise changes in rectal temperature, serum lactic acid, creatine kinase, lactic dehydrogenase, and percentage change in plasma volume. However, the methodology protocol was not of sufficient intensity or duration to produce exhaustion. This study reveals that physiological responses to low to moderately-intensity protocols are not affected by aspartate ingestion.

Maughan and Sadler (1983) investigated the effects of 6 grams per day of aspartate ingestion (versus placebo) on eight healthy young male subjects, who exercised to exhaustion on a bicycle ergometer at a constant workload requiring approximately 75% $VO_{2max}$ using a double-blind protocol. Exhaustion was reached after 82.7 ± 23.5 minutes

(mean ± SD) following aspartate treatment and after 85.4 ± 26.5 minutes following the placebo. There were no significant differences across treatments for blood glucose, lactate, ammonia, or FFA concentrations. As in the Hagan et al. (1982) study, results from this study suggest that aspartate supplementation has no effect on ammonia levels and performance response with moderate exercise.

De Haan et al. (1985) examined the effects of 7.64 g of daily $K^{+}$ $Mg^{+2}$ aspartate supplementation on force production and endurance time for arm exercise. In a series of twelve sessions, twenty healthy male students (19-26 yr) performed arm exercise at 40% of maximal force until exhaustion, and repeated the effort following a 5-minute recovery period. Each session lasted 10 - 15 min. Following session three, the subjects were divided into three groups (control, placebo, and aspartate) for the remaining 9 trials. Results between groups indicated no significant increases in either the exerted force or endurance time. Blood parameters were not reported. Once again, it is speculated that the low relative exercise intensity failed to raise ammonia levels to the point where aspartate could exert a beneficial effect; further, blood ammonia was not reported.

Wesson et al. (1988) investigated high-intensity cycling endurance in response to aspartate intake, and measured blood ammonia, glucose, and FFA. Seven healthy young male athletes ($VO_{2max}$ - 53.1 ml/kg/min) performed leg exercise to exhaustion at a workload ~75% $VO_{2max}$ at 50 rpm on a bicycle ergometer. The test was administered after ingestion of 10g of potassium-magnesium aspartate or placebo over a 24-hour period utilizing a double-blind design. Time to exhaustion significantly ($p < 0.01$) increased by ~14% following aspartate ingestion (87.6 min vs. 75.7 min) vs. placebo. Further, blood ammonia levels were lower in the aspartate condition at rest, during exercise, 15 min post exercise, but not at exhaustion ~ [110 μM/L]. This may indicate an improvement in the efficiency of ammonia removal, or in bioenergetic metabolism to prolong high-intensity

endurance and performance. The aspartate group also had higher ($p < 0.05$) blood [FFA] at exhaustion (0.74 mM vs. 0.60 mM), with no difference at the other time points. Other findings of note were: 1) a higher HR at exhaustion (168 bpm vs. 163 bpm) for the aspartate treatment vs. placebo trial, and 2) a significant reversal 15 min post-exercise with the aspartate condition having a significantly lower HR (90 bpm vs. 98 bpm) vs. placebo. Blood glucose remained stable ~ [5 mM/L] across treatments. The conclusions from this study were that aspartate is an ergogenic aid due to the ammonia lowering effect and the glycogen sparing of increased availability of FFA's.

Work by Denis et al. (1991), and Eto et al. (1994) investigated the effects of other AA combination(s) designed to lower the ammonia response to steady state high-intensity (~80% $VO_{2max}$) cycling. However, in these studies, there was neither an exhaustive component nor a performance measure. In the Eto et al. study (n=3), ingestion of 20g of arginine-glutamate (AG) two hours prior to cycling significantly lowered ($p < 0.01$) blood [$NH_3$] in the AG group at 60 min (98 ug/dl) vs. placebo (67 ug/dl). Resting blood ammonia concentrations were not different across treatments. Conversely, Denis et al. (1991) found no difference in blood [$NH_3$] at 45' of cycling at 80% $VO_{2max}$ following chronic ingestion of 20g of chronic arginine + aspartate (AA) ingestion (5g x QID x 10 days) vs. placebo. Of note, in both of these high-intensity (but steady state) studies, blood ammonia levels did not rise to [100 μM/L]. These results indicate, that in humans, blood [$NH_3$] > 100 μM/L during exercise is not tolerated well by the body, and work stoppage occurs quickly. Furthermore, these human studies also show that when blood $NH_3$ concentrations during high-intensity steady-state work are below 100 μM/L (e.g. 60 – 80 μM/L), work may be tolerated much longer (i.e. hours), without exceeding the critical balance between ammonia buildup, ammonia removal, and onset of fatigue.

Tuttle et al. (1995) investigated the effects of 150 mg/kg of daily aspartate supplementation (versus placebo) on weight lifting performance and blood ammonia, lactate, and RPE response in twelve healthy young men utilizing a double blind, counterbalanced protocol. The exercise testing phases (65% of one repetition maximum, bench press to failure) were administered one week apart, with a week long weight-training regime that consisted of 3-5 sets, at approximately 70% of 1 repetition maximum, for 8 repetitions per set. At the conclusion of the study, no significant differences in blood ammonia concentrations [μM/L] with (aspartate versus placebo) were found at rest, during exercise, or post exercise. Exercise blood ammonia concentrations ranged from 66.3 – 88.5 μM/L with no difference between treatments. As expected, post-exercise blood ammonia concentrations were higher in both groups versus pre-test, however, there was no change in lactate versus baseline with either treatment. There was no difference in the number of repetitions completed (9.5 versus 10.3, respectively) during the exercise test following the aspartate or placebo treatments. These results indicate aspartate supplementation has no effect on blood ammonia concentrations or performance in response to weight training.

Columbani et al. (1999) investigated the effects of 15 grams of arginine aspartate supplemented daily for two weeks before a marathon run in 20 endurance-trained males in a double-blind crossover design. Blood samples were collected at four time points, as follows: 1) before the run, 2) after 31 km, 3) at the end of the run, and 4) during recovery. Results indicated that carbohydrate, fat metabolites, cortisol, and ammonia were unaffected by supplementation. However, plasma somatotropic hormone, glucagon, and urea were significantly increased, and most of amino acids were reduced. The mean running time was 194 minutes (placebo) and 196 minutes (supplement), and not significantly different. This study demonstrated no obvious benefit with chronic arginine-aspartate supplementation and marathon performance.

Ritter et al. (1999) investigated the effects of five consecutive days (4 x 360 mg QD) aspartate supplementation (or placebo) on one mile run time in endurance-trained men. Mean mile run time improved in the aspartate group from 6:01 to 5:53. However, in the placebo group, mean times also improved from 5:47 pre-treatment to 5:43 post treatment. These results suggest that aspartate treatment may result in significant ergogenic benefits in trained athletes, however, this protocol failed to utilize a double blind protocol nor report blood ammonia values to validate the findings. Results from human studies are discussed inclusively with the animal studies in Section 1.6 below.

**1.6 Summary and Conclusions**

The merit of aspartic acid as an ergogenic aid in humans is unresolved. Findings indicate that species, testing modality, testing intensity, training specificity, and training status affect blood ammonia concentrations and physical performance in response to L-Aspartic Acid ingestion. Of these, it appears that exercise intensity and status of training in reference to the testing modality has the greatest influence upon the metabolism of ammonia and aspartate and performance response. The effect of gender is unknown.

Possibly, a more consistent or specific subject pool (i.e. testing subjects in training specific modalities) might reveal a consistent pattern in results or differences between groups. Measurements of plasma catecholemines, cortisol, insulin, glucagon, amino acids, nitrogen metabolism enzymes (SGPT, SGOT, GDH), adenine nucleotides, blood lactate, and ammonia would provide additional metabolic and endocrine data. The review of literature suggests that in order for aspartate supplementation to have an effect on EIH, the exercise intensity should be a minimum of 75% $VO_{2max}$, and total daily dosage of aspartate should be ~ 5 to 15 grams. Finally, there may be unknown factors affecting the results, such as diet, weight, gender, and purity of the amino acid, and protocol of aspartate administration.

As mentioned previously, administration of aspartic acid improves the status and symptoms of clinical fatigue. There are strong indications that aspartate supplementation improves performance in the swimming rat. On the other hand, the effectiveness of aspartate supplementation for reducing EIH and improving exercise performance in humans is inconsistent. The studies demonstrating the most dramatic improvements in humans (Ahlborg et al., 1968; Franz et al., 1977; Gupta and Srivastava, 1973) did not report blood ammonia, FFA, or assess serial nitrogen biochemistry or assess glycogen sparing. On the other hand, Kendrick (1976) and Tangsakul (1977) reported in rats supplemented with 12.5 mg/kg aspartate a 90% increase in swim time to exhaustion and concurrent reductions ammonia and increases in blood urea. In contrast, Matoush et al. (1964), found no effect of aspartic acid salts on swimming performance on rats or dogs, however, the dosages of aspartate in this study were relatively high, i.e. 2000 mg/kg. Subsequent investigations in humans, tried various dosages and protocols, but no consistent successful trends in methodology were established. Endurance protocols (e.g. walking at 62% $VO_{2max}$) low-moderately-intensity arm work (40% of maximal force), weightlifting, and cycling at 75% $VO_{2max}$ have revealed no change in work time to exhaustion following similar doses of aspartate treatment (De Haan et al., 1985; Hagan et al., 1982; Maughan and Sadler, 1983). Further, data from Fallis et al. (1963) and Tuttle et al. (1995) indicates that short-term weight lifting protocols may not elevate ammonia to the point (e.g. > [100 μM/L]) where aspartate ingestion for reduction in hyperammonemia can be effective.

As presented previously in Table 1.3, the anaplerotic roles of aspartate provide clues into the bioenergetic mechanisms for enhanced work capacity with supplementation. As noted by Gibala et al. (1997), ammonia and aspartate metabolism link (i.e. 'anaplerosis') the PNC and TCA cycle. However, as cited by Graham et al. (1997) in reference to the PNC, it is unclear as to "what stimulates the key enzymes, or how the

tissue manages to obtain adequate amounts of aspartate, a key substrate". As evidenced recently (Gibala et al., 1997) in short-term (< 5 min) activity, large increases in succinate, malate, and fumarate (> 90% of the total TCA cycle substrate pool) are observed during the first minute of muscular contraction. Therefore, accelerated activity of the TCA cycle is promoted in short-term intense exercise. Further, Lancha et al. (1995) found increases in pyruvate carboxylase activity (and time to exhaustion) in swimming rats with aspartate supplementation. However, the human studies exhibiting enhancements in performance via aspartate ingestion lack measurements of appropriate blood values (e.g. enzyme, $NH_3$, urea, AA, FFA) to discern the appropriate physiological mechanism(s) (Ahlborg et al., 1968; Franz and Chintanaseri, 1977; Gupta and Srivastava, 1973).

As demonstrated by Lancha et al. (1995) and Wesson et al. (1988) aspartate intake increases FFA utilization during long exhaustive high-intensity (i.e. > 75% $VO_{2max}$) exercise thus sparing muscle glycogen (vs. placebo). Animal studies have utilized swimming as the primary mode, while human studies have concentrated on moderately-intensity long duration stationary cycling and walking, with a further shortage of studies on females and highly-trained subjects. Bicycling studies are the most promising in humans with regard to aspartic acid supplementation and performance, possibly because of the prolonged localized recruitment of Type IIa - IIb muscle fibers at high workloads, promoting high levels of blood $NH_3$.

Summarizing, postulated mechanisms of aspartate supplementation to increase work tolerance include:

1) Reducing blood ammonia
2) Increasing urea synthesis
3) Increasing aspartate substrate in the PNC, promoting the regeneration of AMP and maintenance of total adenine nucleotide levels
4) Providing OAA for the TCA cycle via the transamination of aspartate to OAA (aspartate + α-ketoglutarate <=> OAA + glutamate)
5) Increasing aspartate availability for the urea cycle, thus promoting

conversion into less-toxic urea
6) Providing carbons for fumarate for the TCA cycle, via anaplerotic production by the PNC and urea cycle
7) Increasing glycogen synthesis
8) Increasing FFA availability, thus sparing muscle glycogen (Lancha et al., 1995; Stubbs and Krebs, 1975)
9) Providing substrate for gluconeogenesis (via fumarate, OAA)
10) Shuttling reducing equivalents across the inner mitochondrial membrane for the ETC.

Therefore, the available literature in animals and humans suggests that ~ 5 - 12.5 g x 2 of aspartate ingested over a 24-hour period preceding intense exercise may enhance exercise performance. As shown by Cutinelli et al. (1970), aspartate supplementation promotes the maintenance of AMP (by the PNC), thus enhancing the desirable maintenance of the total adenine nucleotide pool (ATP + ADP + AMP). Further, aspartate may increase the anaplerotic availability of the TCA cycle intermediate fumarate by promoting fumarate formation in both the PNC and urea cycle. Unfortunately, there are limited studies investigating this area during high-intensity exercise performance, when aspartate levels decline and blood ammonia levels continue to rise. Thus, the mechanisms, dosage regime, and potential of aspartate supplementation to improve ammonia clearance and high-intensity exercise performance in humans are unresolved. To address the above concerns, it is hypothesized that 5 - 15 grams of aspartate BID over a 24-hour period preceding high-intensity exercise will decrease blood ammonia levels, and increase short term exhaustive bursts in power.

In summary, although the impact of aspartate supplementation on performance appears reflective of specificity of training, the degree of effectiveness on gender is unclear. Secondly, the reliability of the blood ammonia assay across exercise sessions is unclear as well, as is the dose-response effect of variable L-Aspartic Acid supplementation on exercise hyperammonemia. Third, the effect of aspartate on high-intensity performance and hyperammonemia is unclear comparing well-trained versus lesser-trained men.

Therefore, to test these areas and hypotheses, the dissertation consisted of a preliminary phase of three studies (summarized in the introduction to Chapter 2), followed by a second phase of three experiments (presented in Chapters 2, 3, and 4, respectively). Each experiment utilized an incremental arm ergometer protocol, accompanied by a 24-hour supplementation regime of aspartate or placebo. The purposes of the three experiments were:

Experiment 1:
a) Assessed the reliability of blood ammonia in college-age subjects across five high-intensity exercise testing sessions holding duration and power constant
b) Assessed the blood ammonia response in college-age subjects to aspartate intake vs. placebo, counterbalanced across five high-intensity exercise sessions holding duration and power constant

Experiment 2: Assessed high-intensity exercise performance over two sessions in highly-upper body trained and moderately upper body trained college-age men, following aspartate supplementation or placebo, utilizing a double blind counterbalanced design.

Experiment 3: Assessed high-intensity exercise performance over two sessions in moderately upper body trained college-age women, following aspartate supplementation or placebo, utilizing a double-blind counterbalanced design.

# CHAPTER 2. PRELIMINARY STUDIES OF BLOOD AMMONIA REDUCTION AGENTS AND THE RELIABILITY OF THE AMMONIA ASSAY ACROSS EXERCISE SESSIONS

## 2.1 Introduction and Rationale

Short-term and long-term decreases in blood ammonia concentration are associated with improvements in high-intensity exercise performance (Wesson et al., 1988; Graham et al., 1995). Assessment of blood [$NH_3$] during high-intensity exercise is becoming an increasingly prevalent marker for 1) status of activity-specific conditioning, 2) protein and amino acid metabolism, 3) degree of effort and 4) assessment of performance. Furthermore, the symptoms of high-intensity exercise-induced hyperammonemia (i.e. ataxia, dizziness, confusion, nausea), mimic that of clinical hyperammonemia where blood [$NH_3$] exceeds 100 μM/L (Batshaw, 1994). The reduction of hyperammonemia, and associated symptoms, is desirable for optimal physiological function and athletic performance.

Data from Barnes et al. (1964), Kendrick (1976), and Wesson et al. (1988), suggest that reduced hyperammonemia and improved exercise performance are in some cases, associated with the acute intake (~5 - 15 grams) of the ammonia reducing L-amino acids (e.g. aspartate, arginine, ornithine, glutamate). However, there is insufficient information on the 1) overall reliability of the blood ammonia assay across multiple exercise sessions, and 2) dose-response effect of amino acid ingestion (e.g. aspartic acid) on blood ammonia reduction to validate the blood ammonia measurement with high-intensity exercise. Therefore, the purpose of this chapter is to 1) summarize three pilot studies investigating blood ammonia reduction and high-intensity performance with ingestion of the ammonia-reducing amino acids, providing background and rationale for 2) a reliability investigation of the blood ammonia assay across exercise sessions.

### 2.1.1 Summary of Pilot Work

Reliability studies involving invasive biochemical assays, high-intensity exercise, dietary control, and humans are difficult to accomplish with an adequate number (i.e. $n \geq 10$) of subjects due to 1) the invasiveness of blood draws and/or muscle biopsies, 2) the required control of diet, caffeine, tobacco, exercise habits, and other factors, 3) the high dropout rate, 4) the higher likelihood of an adverse physiological or medical event, and 5) the intrinsic subject motivation to complete the study. In this regard, publications in exercise biochemistry often appear with sample sizes considered 'smaller than usual' (i.e., $n < 6$) in contrast to non-exercise biochemistry disciplines. Consequently, publications of this type (e.g. the reliability of the blood ammonia assay during exercise across time) of exercise physiology research are rare, of higher risk, and difficult to conduct. However, biochemical reliability investigations are necessary to validate potential biochemical changes in response to treatment. With this in mind, three pilot studies each with sample sizes < 10 were conducted (preceding a more thorough reliability investigation) to assess the potential trends of selective amino acid ingestion to lower blood ammonia and/or increase blood urea and performance during high-intensity exercise.

The first pilot study (i.e. pilot study 1, double-blind) investigated the effects of supplementation of 2g each of glutamate, arginine, ornithine, and aspartate (versus placebo) on high-intensity leg ergometry performance, resting and post-exercise blood [$NH_3$], lactate, and urea in moderately-trained college-age males (n = 3). With treatment, there was a significant drop in blood [$NH_3$], an increase in urea, and no significant change in lactate or performance in response to treatment (For detailed methodology and results, see appendix B.).

A second pilot study (i.e. pilot study 2) in healthy college students (n = 8) isolated the effects of ingestion of 10g each of arginine and ornithine (urea cycle substrates) on

resting and post-exercise blood $NH_3$, lactate, creatinine, total protein, urea and time to exhaustion in response to incremental treadmill exercise. Results indicated ~ 40% increase in urea with treatment (versus placebo), with no significant differences in blood ammonia, LA, total protein, creatinine, or time to exhaustion. These results suggest 1) isolated urea cycle substrate ingestion has a greater effect on blood urea elevation than on blood $NH_3$ reduction, and 2) high-intensity performance is not affected by short-term ingestion of isolated urea cycle amino acids (For detailed methodology and results, see appendix C.).

A third pilot study (i.e. pilot study 3) investigated the effect of variable dosage of aspartate (0, 5, 10, 15g) on immediate post-exercise blood $NH_3$ and lactate in surfers (n = 5). Work time and power output were constant per subject across four exercise sessions utilizing an incremental arm ergometer protocol. Results indicated a dose-dependent drop in blood $NH_3$ in response to L-Aspartic Acid supplementation, with no change in lactate (For detailed methodology and results, see appendix D.). Results from this study indicated 10 - 15g L-Aspartic Acid prior to intense arm-crank activity as the optimal dose (of 0, 5, 10, or 15g) to lower blood ammonia concentration in moderately-trained subjects. However, yet to be resolved from these pilot studies and previous work with the reduction of exercise-induced hyperammonemia and improvement in performance with aspartate supplementation (Barnes et al., 1964; Kendrick, 1976; and Wesson et al., 1988) is the reliability of the blood ammonia assay across exercise sessions under placebo and/or L-Aspartic Acid treatment.

Therefore, to 1) assess the reliability of the blood ammonia assay under either placebo or L-Aspartic Acid condition(s) across exercise sessions, and 2) validate the pilot study results of blood ammonia reduction with L-Aspartic Acid treatment; and 3) provide reference for future exercise-induced hyperammonemia studies, the following reliability experiment (with sample size $\geq$ 10) was conducted.

## 2.2 Methods

### 2.2.1 Subjects

Fifteen recreationally upper body trained (e.g. weight training, surfing, swimming) college-age male (n = 11) and female (n = 4) students attending Brigham Young University – Hawaii (BYU-H) volunteered for the study, approved by the Institutional Review Board of the university. Subjects were informed of the nature of the study, the possible risks associated with participation, and of their right to withdraw at any time before providing written consent. Subject characteristics (mean, SEM, SD, minimum, and maximum) for these fifteen students are presented in Table 2.1. Subjects were instructed to abstain from alcoholic beverages, caffeine, and tobacco for the duration of the study and to keep dietary and exercise habits consistent across sessions. All reported at least one year of experience of upper body recreational training 2-3x/week (i.e. weight training, surfing, swimming). None were members of intercollegiate athletic teams. Training status was measured in wattage ($W \cdot min^{-1}$) at an RPE of 17 (6-20 scale) on an incremental arm ergometer protocol.

### 2.2.2 Protocol

A counterbalanced latin square design determined the order of L-Aspartic Acid (A) or placebo (P) supplementation prior to five exercise sessions. Figure 2.1 illustrates regimen for supplementation assignment. Subjects were assigned to either protocol (a), (b), or (c) in that order, upon registration. Two sessions followed 12.5g x 2 QD of L-Aspartic Acid supplementation, while three followed placebo. A 48-hour washout separated each session. Of the initial pool of 25 registrants, 15 completed at least four sessions, which included three placebo sessions. Of the 15 subjects, seven were assigned to protocol (a), four to protocol (b), and four to protocol (c). Due to technical difficulties, bloodwork was available for only ten subjects who completed the two sessions of L-Aspartic Acid supplementation. Therefore, complete blood ammonia data was available for fifteen

Table 2.1. Descriptive characteristics of the subjects (n = 15) in experiment 1.

| Subject Initials | Subject # (M/F) | Age (yr) | Height (cm) | Weight (kg) | Dosage (mg/kg) | $W \cdot min^{-1}$ @RPE17 |
|---|---|---|---|---|---|---|
| NN | 1F | 20 | 167.6 | 59.1 | 211.5 | 75.0 |
| CS | 2F | 22 | 167.6 | 57.3 | 218.3 | 125.0 |
| JS | 3F | 22 | 162.6 | 68.2 | 183.3 | 100.0 |
| KU | 4M | 23 | 193.0 | 88.6 | 141.0 | 150.0 |
| AH | 5F | 20 | 165.1 | 52.3 | 239.1 | 100.0 |
| NG | 6M | 22 | 170.2 | 68.2 | 183.3 | 150.0 |
| JB | 7M | 22 | 172.7 | 77.3 | 161.8 | 175.0 |
| KN | 8M | 22 | 170.2 | 68.2 | 183.3 | 150.0 |
| MR | 9M | 22 | 172.7 | 77.3 | 161.8 | 175.0 |
| JA | 10M | 24 | 182.9 | 85.5 | 146.3 | 200.0 |
| JS | 11M | 22 | 175.3 | 75.0 | 166.7 | 200.0 |
| DN | 12M | 24 | 170.2 | 66.8 | 187.1 | 150.0 |
| JE | 13M | 22 | 180.3 | 83.2 | 150.3 | 150.0 |
| SA | 14M | 33 | 180.3 | 94.1 | 132.9 | 150.0 |
| YN | 15M | 27 | 177.8 | 70.5 | 177.4 | 175.0 |
| Mean | | 23.1 | 178.4 | 73.8 | 171.3 | 158.3 |
| SEM | | 0.8 | 2.6 | 2.9 | 6.6 | 9.3 |
| SD | | 3.2 | 7.8 | 8.7 | 19.7 | 28.0 |
| Minimum | | 20.0 | 170.2 | 61.4 | 141.0 | 100.0 |
| Maximum | | 33.0 | 193.0 | 88.6 | 203.7 | 200.0 |

RPE - Rating of perceived exertion (6-20) scale
mg/kg – Relative dosage per kg body weight of 12.5 grams of L-Aspartic Acid

sessions following placebo, and ten sessions following L-Aspartic Acid supplementation. Two drink powder packets (either two of A, or two of P) were distributed to the subject(s) 24 hours prior to each session. Subjects were instructed to mix the contents of each in 8 - 12 ounces of water, and ingest one packet mixed in water 24 hours and ingest the other packet 1 hour before testing, respectively. Of the five sessions, two followed ingestion of A, while three followed ingestion of P. Contents of packet A were 12.5g of L-Aspartic Acid (Source Naturals, Inc., Scotts Valley, CA) mixed in 20g of powdered Powerade© (The Minute Maid Company for Coca-Cola USA, Northlake, IL). Packet P contained

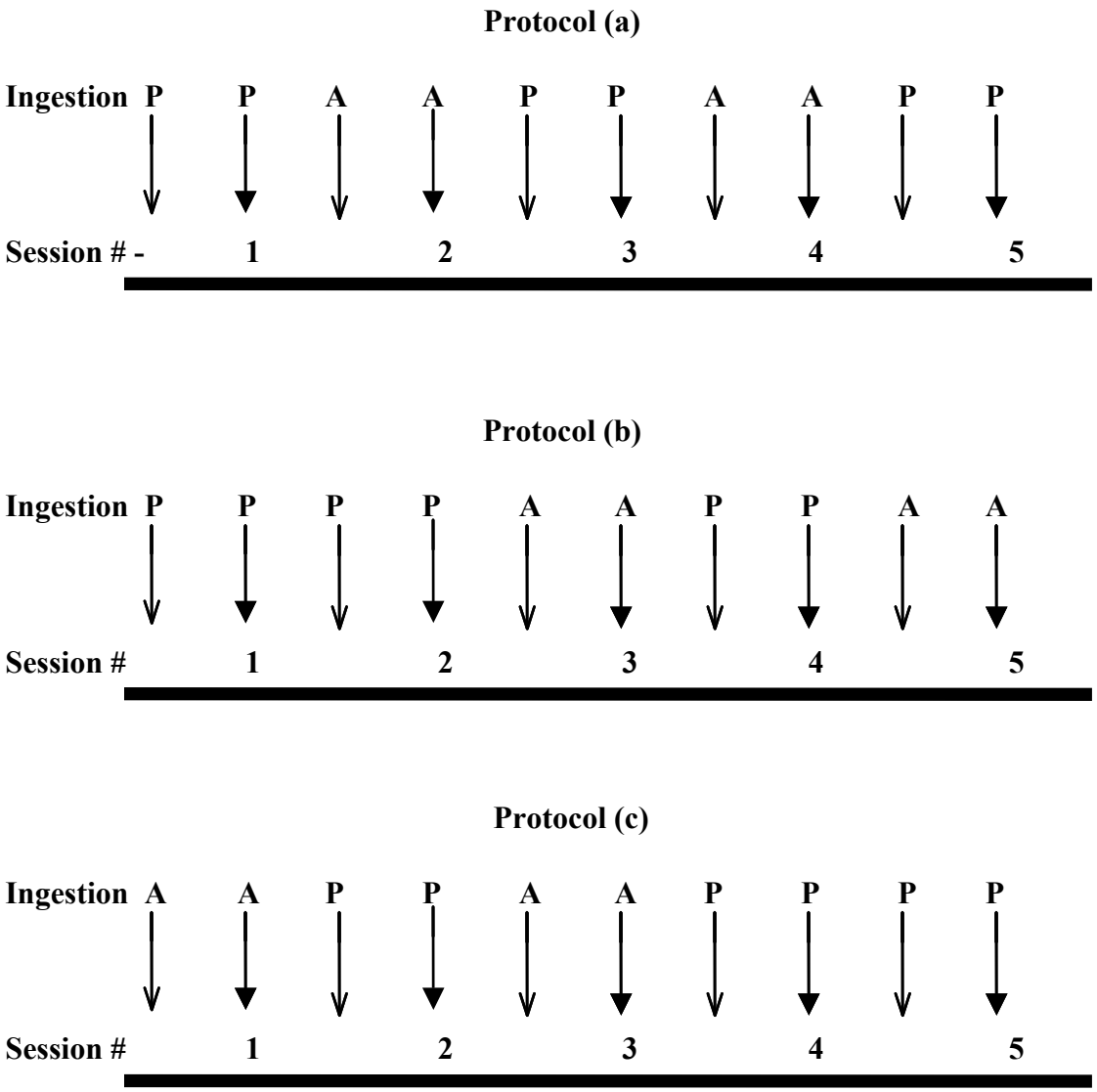


Figure 2.1. Order and regime of three counterbalanced protocols for supplementation assignment (P – placebo; A – L-Aspartic Acid) across five exercise sessions. Subjects were assigned to protocol (a), (b), or (c) in the order of signup for participation. (⟶ = ingestion ~ 24 hours pre-test; ➡ = ingestion ~ 1 hour pre-test)

20g of powdered Powerade© only. Powders were weighed to within 0.1g on a VWR Sargent Welch Calibrated Scale (VWR Scientific Products #WLS2648-30). Staff instructions for distributing L-Aspartic Acid (A) or placebo (P) packets packet(s) to subjects are presented in Appendix D.

Subjects reported to the laboratory for five sessions of seated incremental arm ergometry (Monark Model #881) testing. At least 48 hours separated each session. Testing began at 25 $W \cdot min^{-1}$, and increased in 25 $W \cdot min^{-1}$ increments every 30 seconds at a constant 100 rpm. The test was terminated upon stage completion when rating of perceived exertion of 17 (6-20 scale) was achieved. The individual duration time achieved in session 1 was recorded, and all subsequent tests went the same length of time with the same workload ramp. Cadence and revolutions were monitored and tracked, respectively, via an integrated electronic readout. Verbal feedback was used to help subjects stay at target cadence. Revolutions achieved were used to calculate wattage output. The intra-subject exercise protocol duration time and number of stages completed was kept the same across sessions. Inter-subject number of stages completed varied. Protocol duration, total revolutions, and mean terminal wattage in the RPE 17 stage ($W \cdot min^{-1}$) were tabulated and recorded.

Seated blood samples were drawn 3 minutes post exercise. Following alcohol preparation of skin, a 21 gauge 1" Vacutainer© (Becton Dickinson and Co. Vacutainer Systems, Franklin Lakes, NJ) double-sided blood collection needle (#36-7212) was inserted into a superficial antecubital vein. A red top Vacutainer© tube with sterile interior was then inserted into the surrounding needle hub, and a 4-6 ml blood sample drawn for about 15 seconds. Blood samples were placed immediately into an ice bath for 10 minutes, and centrifuged for 10' at 3400 rpm (Fisher Scientific Centrific Model 228). Serum was transferred via disposable 3.2 ml polyethylene transfer pipettes (#13-711-7, Fisher

Scientific, Pittsburgh, PA) into labeled 1.5 ml micro polypropylene metal-free test tubes (# 223-9480, Bio-Rad Laboratories, Hercules, CA), sealed with attached caps, and frozen immediately at 20 degrees Celsius for subsequent batch analysis. Serum [$NH_3$] was determined via a Kodak Ektachem Analyzer (Eastman Kodak Company, Rochester, NY) using Ektachem $NH_3$/Amon Clinical Chemistry Slides (Johnson and Johnson Clinical Diagnostics, Rochester, NY) using a phenol hypochlorite colorimetric technique.

### 2.2.3 Statistical Analyses

The SPSS statistical package was used to assess the intraclass correlation coefficient (ICC) between placebo sessions 1,2, and 3 (i.e. P1, P2, P3) and between the two L-Aspartic Acid sessions (i.e, A1, A2). An analysis of variance with repeated measures was used to assess differences between sessions of placebo treatment (P1, P2, and P3) and L – Aspartic Acid supplementation (A1 and A2), using GB-STAT software. Tukey's post-hoc t-tests were utilized when the overall F value was significant. Individual intraclass correlation coeffecients were calculated also between the individual placebo sessions (P1 vs. P2, P1 vs. P3, and P2 vs. P3), and between the L-Aspartic Acid supplementation sessions (A1 vs. A2). $\alpha$ was set *apriori* at .05.

## 2.3 Results

As specified per analyses, results are presented as the actual value, or as mean ± SEM. Figure 2.2 illustrates the consistency of the individual blood ammonia responses [μM/L] across the three exercise sessions with placebo treatment. Figure 2.3 illustrates the individual blood ammonia responses [μM/L] to two exercise sessions with L-Aspartic Acid supplementation. The ICC was highly significant ($r = 0.90$, $p < 0.0001$) for the blood ammonia concentrations [μM/L] across the three placebo sessions. The intraclass correlation coefficient between the two sessions for L-Aspartic Acid treatment was also significant, $r = 0.76$, $p < 0.01$. The individual intraclass correlation coefficients

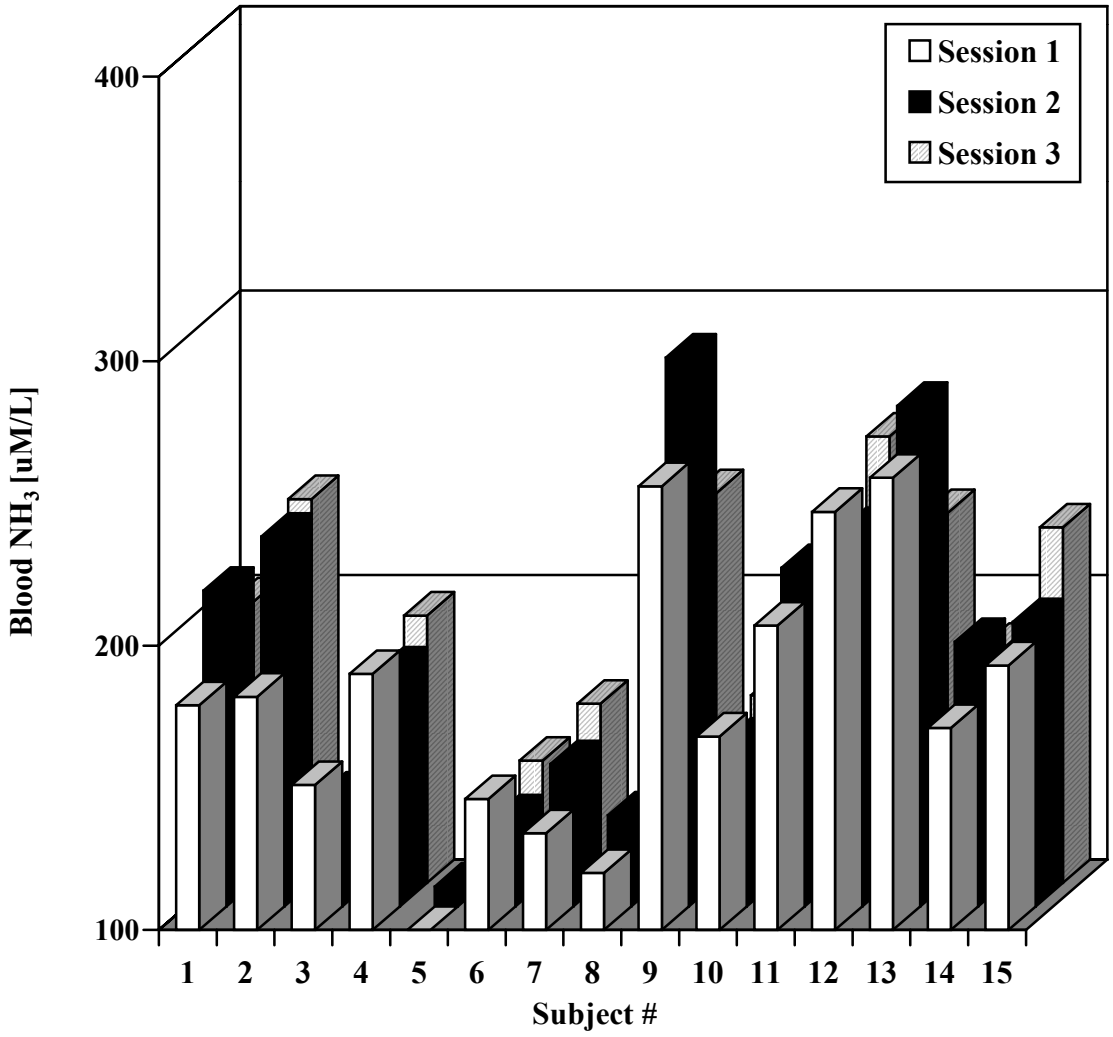


Figure 2.2. Individual responses in blood $NH_3$ [μM/L] following treatment with placebo across three exercise sessions (n = 15).

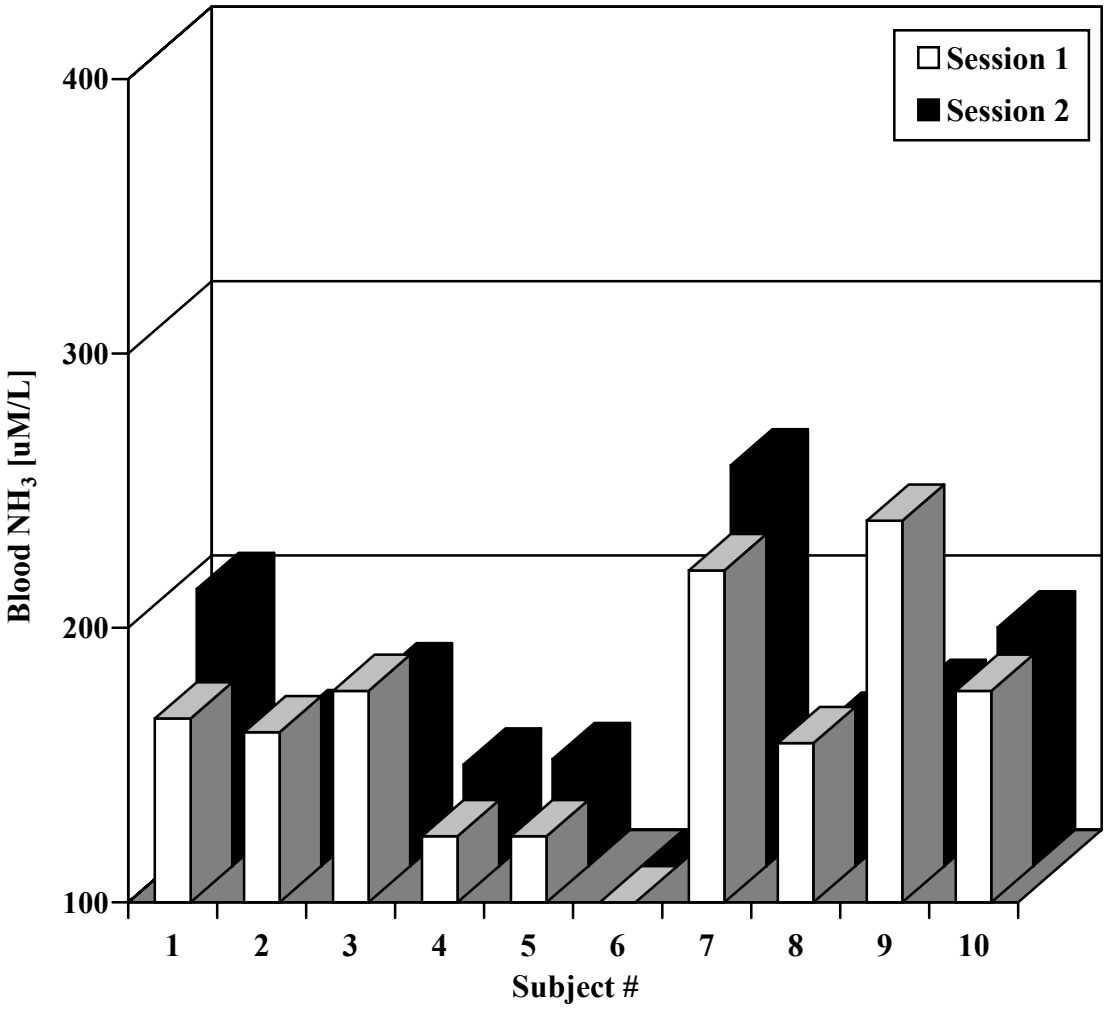


Figure 2.3. Individual responses in blood $NH_3$ [μM/L] following treatment with L-Aspartic Acid across two exercise sessions (n = 10).

between P1, P2, and P3 were also highly significant (P1 vs. P2, $r = 0.91$, $p < 0.0001$; P2 vs. P3, $r = 0.90$, $p < 0.0001$; P1 vs. P3, $r = 0.89$, $p < 0.0001$). There were no significant within-group differences ($p > 0.05$) between the placebo sessions or the L-Aspartic Acid sessions.

Figure 2.4 illustrates the significantly reduced blood ammonia concentrations with the sessions of L-Aspartic Acid treatment versus placebo ($F = 25.6$ $p < 0.0001$). The means ± SEM of blood [$NH_3$] across the treatments were: P1 = 180 ± 13, P2 = 190 ± 14; and P3 = 184 ± 13, and A1 = 165 ± 14, and A2 = 163 ± 14. There were no significant differences ($p > 0.05$) in [blood ammonia] between P1, P2, and P3, or between A1 and A2. The lowest blood [$NH_3$] per subject was with L-Aspartic Acid supplementation.

**2.4 Discussion**

This investigation is the first to report the test-retest reliability of the blood ammonia assay across high-intensity exercise testing sessions. Results indicate that the urease/phenol hypochlorite method (with the Kodak© Ektachem Vitros II Analyzer) is highly reproducible across three separate testing days in a controlled environment with recreationally-trained college-age males and females. However, until future information becomes available, one should exercise caution for application across additional testing methods, dietary fluctuations, subject populations, and/or exercise habits. These results show the blood ammonia assay to be highly reliable (i.e., $r \geq .90$, $p < 0.0001$) across multiple exercise sessions with placebo treatment in the short term (i.e. < two weeks). The present investigation suggests also a greater variability in the reproducibility of the blood ammonia assay with L-Aspartic Acid supplementation. Although statistically significant, the reliability of the ammonia assay across exercise sessions with L-Aspartic Acid supplementation is less significant (i.e. $r = 0.74$, $p < 0.05$) than that of placebo. This difference may be due to greater aspartate intake in foodstuffs (e.g. meat), and/or intra- and intersubject variability in metabolic aspartate bioefficiency associated with training status.

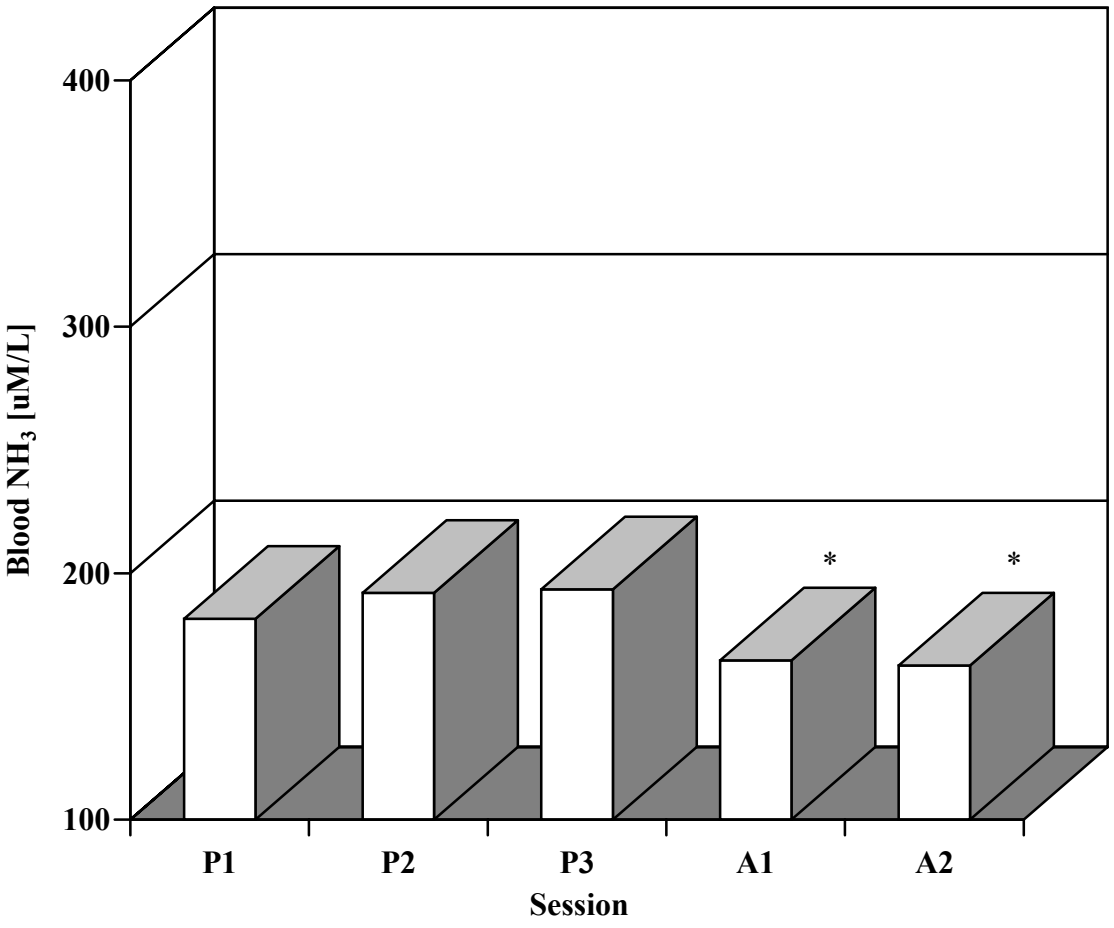


Figure 2.4. Mean blood $NH_3$ [μM/L] across three placebo sessions (P1, P2, P3) and two L-Aspartic Acid treatment sessions (A1, A2).
*$p < 0.05$, (A1, A2 versus each of P1, P2, P3)

For instance, it is well known that moderately-trained subjects (similar to those trained in this study) have reduced ammonia clearing capabilities versus that of highly-trained subjects (Graham et al., 1997). Although the sample sizes in the present study differed between the two treatments (i.e., placebo, n = 15 x 3; L-Aspartic Acid, n = 10 x 2), the blood ammonia concentrations were significantly lower (as hypothesized) with L-Aspartic Acid supplementation versus placebo, while keeping intra-subject power output constant across sessions.

To conclude, these results show the blood ammonia assay to be a highly reproducible short-term tool across exercise sessions under controlled conditions. However, until sufficient information becomes available, one should exercise caution when applying these results to subject groups undergoing changes in diet or training status. Further, though significantly reproducible but more variable versus placebo, this investigation provides evidence also that the blood ammonia assay is a reliable measurement in exercise studies utilizing L-Aspartic Acid supplementation, particularly with recreationally-trained college students. To apply this reproducibility to other subject pools, regimes of amino acid supplementation, dietary or testing protocols, and/or long term changes in training statuses needs to be investigated further.

# CHAPTER 3. IMPACT OF L-ASPARTIC ACID SUPPLEMENTATION ON EXERCISE HYPERAMMONEMIA AND PERFORMANCE IN HIGHLY-TRAINED AND MODERATELY-TRAINED MEN

## 3.1 Introduction

Ammonia, a product of dietary and exercise metabolism, is inversely associated with exercise performance. Symptoms of clinical hyperammonemia, such as those described by Batshaw (1984) (e.g.. nausea, ataxia, dizziness), mimic those observed during intense exercise, where normal resting blood [$NH_3$] of ~10-40 μM/L may exceed 500 μM/L (Fischbach, 1988). Data from Barnes et al. (1964), Kendrick (1976), and Wesson et al. (1988) indicate that acute intake (~5 - 15 grams over 24 hours) of the non-toxic amino acid aspartate is among the techniques known to reduces exercise-induced hyperammonemia and in some cases improves exercise performance. However, the effect of aspartate on performance is unknown comparing well-trained versus lesser-trained men. Therefore, this experiment was conducted to investigate the effect of aspartate ingestion on high-intensity exercise hyperammonemia and performance in highly-trained and moderately-trained men.

Strenuous physical activity elevates blood ammonia concentrations primarily by accelerating activity of the purine nucleotide cycle (i.e. AMP deaminase) and deamination of branched chain amino acids (Lowenstein, 1972, MacLean et al., 1996). As determined by Lo and Dudley (1987) and Levin et al. (1969), blood ammonia levels are inversely associated with exercise training status and performance, and rise during intense exercise to levels included in the diagnostic criteria (i.e. > [100 μM/L]) for clinical hyperammonemia. The undesirable symptoms of exacerbated clinical hyperammonemia (i.e. oncoming nausea, ataxia, dizziness) are likewise observed during intense exercise, where a normal resting blood [$NH_3$] of ~10-40 μM/L rises to well over [100 μM/L].

The non-essential amino acid L-Aspartate is one of several amino acids (Najarian et al., 1958) known to reduce the hyperammonemia associated with hepatic disorders, and, in

some instances improve exercise performance in healthy populations (Ahlborg et al., 1968; Gupta et al., 1973; Wesson et al., 1988). Though controversial, a review of literature of the past 40 years indicates that an acute reduction in transient hyperammonemia in trained individuals may enhance performance during high-intensity exercise, where blood ammonia levels peak. The regimen for supplementation at present indicates dosage QD between 5 - 15 grams over 24 hours to be most effective; however due to a myriad of testing modalities, subject characteristics, and dosage regimes the acute application of L-Aspartic Acid to reduce exercise-induced hyperammonemia (EIH) to enhance high-intensity performance is unclear (Gupta et al., 1973; Wesson et al., 1988).

This study investigated the hypothesis that acute aspartic acid intake will 1) lower hyperammonemia and 2) improve high-intensity arm-crank power in both highly upper body trained intercollegiate male water polo players and moderately upper–body trained college students.

## 3.2 Methods

### 3.2.1 Subjects

Nine highly upper body trained intercollegiate water polo players (WP) and nine moderately upper body trained (MOD) college-age male students attending Brigham Young University – Hawaii (BYU-H) volunteered for the study, which had the approval of the Institutional Review Board of the university. Subject characteristics (mean, SEM, SD, minimum, and maximum) for WP and MOD are presented in Table 3.1 and Table 3.2, respectively. Subjects were fully informed of the nature of the study, the possible risks associated with participation, and their right to withdraw at any time. Their understanding of this was provided by written informed consent. WP consisted of in-season intercollegiate water polo athletes, who supplemented daily swim workouts with weight training 3-5x/week. MOD subjects were recreationally-trained in activities including

Table 3.1. Descriptive characteristics for intercollegiate water polo (WP) players (n = 9).

| Subject Initials | Subject # (M/F) | Age (yr) | Height (cm) | Weight (kg) | Dosage (mg/kg) | $W \cdot min^{-1}$ @end of warm-up |
|---|---|---|---|---|---|---|
| CS | 1 | 18 | 180.3 | 79.5 | 157.1 | 200.0 |
| CA | 2 | 18 | 180.3 | 84.1 | 148.6 | 200.0 |
| JB | 3 | 18 | 182.9 | 84.1 | 148.6 | 200.0 |
| KD | 4 | 18 | 172.7 | 65.9 | 189.7 | 200.0 |
| RS | 5 | 18 | 190.5 | 72.7 | 171.9 | 200.0 |
| SP | 6 | 18 | 180.3 | 77.3 | 161.8 | 200.0 |
| VM | 7 | 19 | 190.5 | 93.2 | 134.1 | 200.0 |
| AW | 8 | 23 | 180.3 | 84.5 | 147.8 | 200.0 |
| DY | 9 | 18 | 180.3 | 84.1 | 148.6 | 200.0 |
| Mean | | 18.7 | 182.0 | 80.6 | 156.5 | 200.0 |
| SEM | | 0.6 | 1.8 | 2.6 | 5.4 | 0.0 |
| SD | | 1.7 | 5.5 | 7.9 | 16.3 | 0.0 |
| Minimum | | 18.0 | 172.7 | 65.9 | 134.1 | 200.0 |
| Maximum | | 23.0 | 190.5 | 93.2 | 189.7 | 200.0 |

mg/kg – relative dosage of 12.5 grams of L-Aspartic Acid per kg body weight

Table 3.2. Descriptive characteristics for moderately-trained (MOD) subjects (n = 9).

| Subject Initials | Subject # (M/F) | Age (yr) | Height (cm) | Weight (kg) | Dosage (mg/kg) | $W \cdot min^{-1}$ @RPE 17 |
|---|---|---|---|---|---|---|
| AM | 1 | 22 | 185.42 | 72.7 | 171.9 | 150.0 |
| CJ | 2 | 23 | 172.72 | 61.4 | 203.7 | 100.0 |
| EM | 3 | 24 | 180.34 | 71.4 | 175.2 | 175.0 |
| DN | 4 | 23 | 193.04 | 88.6 | 141.0 | 150.0 |
| RO | 5 | 20 | 177.80 | 72.7 | 171.9 | 175.0 |
| DN | 6 | 24 | 170.20 | 66.8 | 187.1 | 150.0 |
| JA | 7 | 24 | 182.90 | 85.5 | 146.3 | 200.0 |
| NG | 8 | 22 | 170.20 | 68.2 | 183.3 | 150.0 |
| JB | 9 | 22 | 172.70 | 77.3 | 161.8 | 175.0 |
| Mean | | 22.7 | 178.4 | 73.8 | 171.3 | 158.3 |
| SEM | | 0.4 | 2.6 | 2.9 | 6.6 | 9.3 |
| SD | | 1.3 | 7.8 | 8.7 | 19.7 | 28.0 |
| Minimum | | 20.0 | 170.2 | 61.4 | 141.0 | 100.0 |
| Maximum | | 24.0 | 193.0 | 88.6 | 203.7 | 200.0 |

RPE - rating of perceived exertion (6-20) scale
mg/kg – relative dosage of 12.5 grams of L-Aspartic Acid per kg body weight

tennis, swimming, surfing, and weight training. Pertaining to diet, subjects were instructed to abstain from alcoholic beverages, caffeine, and tobacco for the duration of the study, and further, to keep dietary and exercise habits consistent across sessions. Training status was assessed by wattage ($W \cdot min^{-1}$) achieved at an RPE of 17 (6-20 scale) on an incremental arm ergometer protocol.

Arm ergometry was chosen as the testing modality because it was specific to the upper body training status of the subjects. In addition, arm ergometry involved a smaller muscle mass, and therefore less endurance and less lab time. Further, testing via arm crank limited the confounding factors, which might have been observed due to inter-individual variations in day-to-day leg activity patterns.

### 3.2.2 Protocol

A counterbalanced double blind design determined order of L-Aspartic Acid (A) or placebo (P) supplementation, while a third party distributed two drink powder packets to the subject(s) prior to each session. Subjects were instructed to mix the contents in 8 ounces of water, and ingest one packet 24 hours and the second packet 1 hour prior to testing. One session followed ingestion of 12.5g x 2 of aspartate (Source Naturals, Inc., Scotts Valley, CA) mixed in 20g of powdered Powerade© (The Minute Maid Company for Coca-Cola USA, Northlake, IL), while the other session followed ingestion of 20g of powdered Powerade© only. Powders were weighed to within 0.1g on a VWR Sargent Welch Calibrated Scale (VWR Scientific Products #WLS2648-30).

Subjects reported to the laboratory for two sessions of incremental seated arm ergometry (Monark Model #881). At least 48 hours separated each session. The exercise protocol consisted of two parts: 1) an incremental warm-up at a constant 100 rpm immediately followed by 2) all-out performance (sprint to volitional exhaustion). The purpose of the warm-up was to have the subject achieve an RPE of 17 (very hard) to

precede the sprint. The warm-up consisted of a series of 30-second stages, at an initial intensity of 25 $W \cdot min^{-1}$, with an increase of 25 $W \cdot min^{-1}$ each stage. The all-out power test to exhaustion began at the 4-minute mark for WP, and at completion of the RPE 17 (6-20 scale) stage for MOD. Warm-up duration across sessions was kept the same intra-subject, while inter-subject the warm-up duration varied for MOD. Cadence (rpm) was monitored during warm-up by both technician and subject via electronic readout to maintain the correct cranking rate. Rpm and total revolutions were monitored and tracked, respectively, via an integrated electronic readout. Verbal feedback was used to help subjects stay at target cadence during warm-up. The warm-up portion ended, and the performance phase began, when the subject completed the stage in which the RPE of 17 was attained. At that point, with a countdown of 5-4-3-2-1, at a command of "GO!", the subjects turned the crank "as fast as possible for as long as possible" until volitional exhaustion where cadence, total revolutions, and time achieved were not visible to the subject. Warm-up duration (seconds), performance duration (seconds), warm-up revolutions, and performance revolutions were tabulated, and from this data, warm-up and performance power ($W \cdot min^{-1}$) were calculated via:

$$W \cdot min^{-1} = (\text{standard ergometer wattage} / \text{standard revolutions}) \times (\text{revolutions} / \text{seconds}) \times (60 \text{ seconds} / 1 \text{ minute})$$

Seated blood samples were drawn 3 minutes post exercise. Following alcohol preparation of skin, a 21 gauge 1" Vacutainer© (Becton Dickinson and Co. Vacutainer Systems, Franklin Lakes, NJ) double-sided blood collection needle (#36-7212) was inserted into a superficial antecubital vein. A red top Vacutainer© tube with sterile interior was then inserted into the surrounding needle hub, and a 4-6 ml blood sample drawn for about 15 seconds. Blood samples were placed immediately into an ice bath for 10 minutes,

and centrifuged for 10' at 3400 rpm (Fisher Scientific Centrific Model 228). Serum was transferred via disposable 3.2 ml polyethylene transfer pipettes (#13-711-7, Fisher Scientific, Pittsburgh, PA) into labeled 1.5 ml micro polypropylene metal-free test tubes (# 223-9480, Bio-Rad Laboratories, Hercules, CA), sealed with attached caps, and frozen immediately at 20 degrees Celsius for subsequent batch analysis. Serum [$NH_3$] were determined via a Kodak Ektachem Analyzer (Eastman Kodak Company, Rochester, NY) using Ektachem $NH_3$/Amon Clinical Chemistry Slides (Johnson and Johnson Clinical Diagnostics, Rochester, NY) using a phenol hypochlorite colorimetric technique.

### 3.2.3 Statistical Analyses

Performance power ($W \cdot min^{-1}$) and blood [$NH_3$] were compared across treatments and groups using a 2 x 2 ANOVA with repeated measures, with post-hoc differences determined using Tukey's test. All statistical analyses were conducted using GB-STAT software. Significance was considered at $p < 0.05$.

## 3.3 Results

As specified per analyses, results are reported as the mean ± SEM, the actual value, the absolute change, or the percentage change in response to treatment. Figure 3.1 shows the individual blood ammonia concentrations [μM/L] following exercise in response to L-Aspartic Acid (L-Asp) supplementation or placebo in intercollegiate water polo players (WP). Eight of the 9 of WP, or 89% demonstrated lower blood ammonia concentration with L-Aspartic Acid supplementation (versus placebo). Figure 3.2 shows individual blood ammonia concentrations [μM/L] following exercise in response to L-Aspartic Acid (L-Asp) or placebo in moderately-trained college males (MOD). As observed with WP, eight of the 9 of MOD, or 89% demonstrated lower blood ammonia concentration with L-Aspartic Acid supplementation.

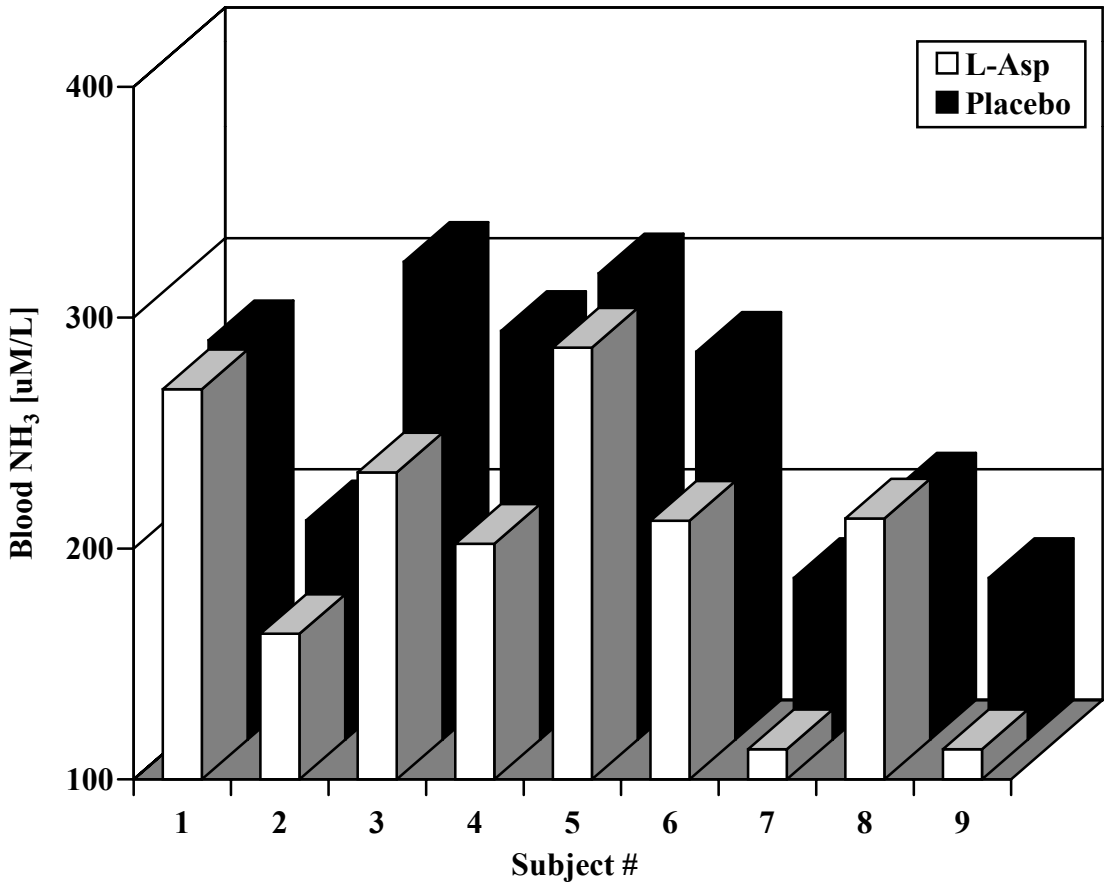


Figure 3.1. Individual responses in blood $NH_3$ [μM/L] following treatment with either L-Aspartic Acid (L-Asp) or placebo in intercollegiate water polo players (n = 9).

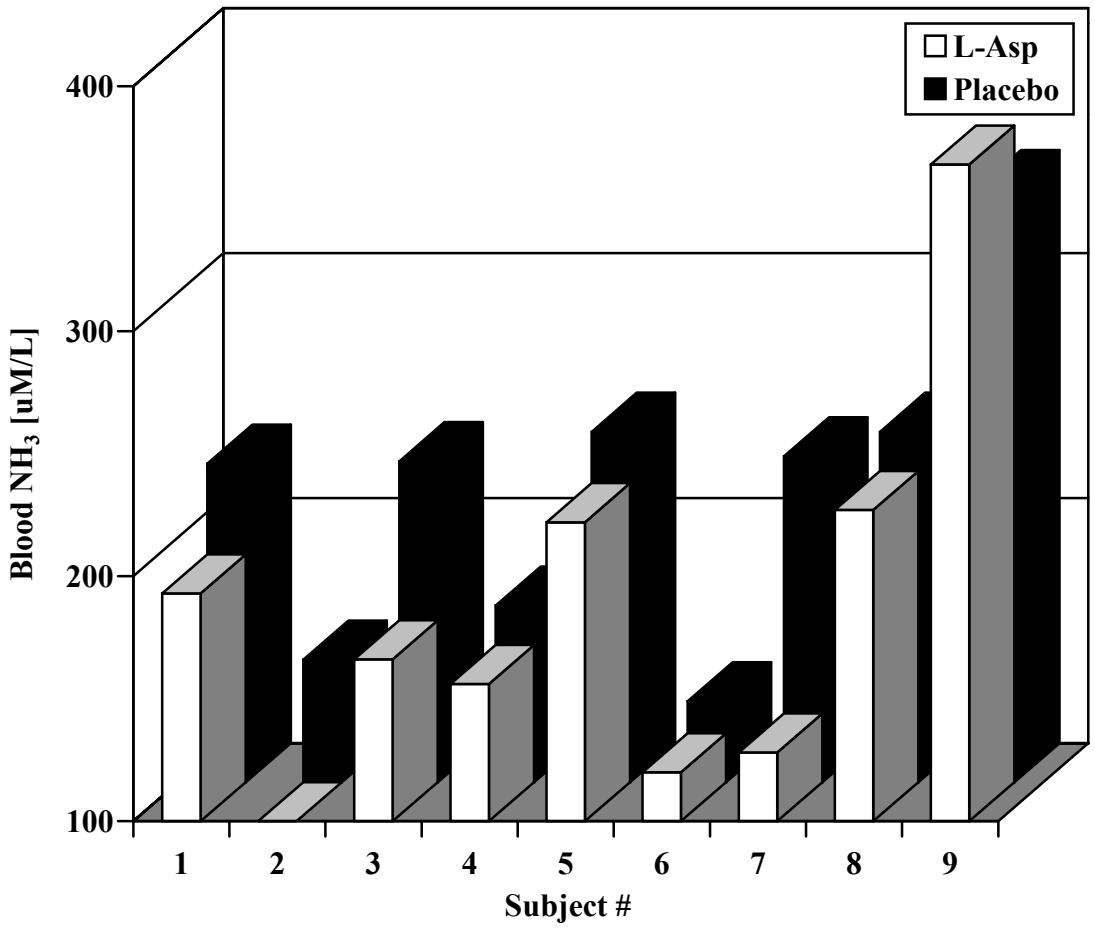


Figure 3.2. Individual responses in blood $NH_3$ [μM/L] following treatment with either L-Aspartic Acid (L-Asp) or placebo in moderately-trained college-age males (n = 9).

Figure 3.3 illustrates the individual responses in performance power ($W \cdot min^{-1}$) to L-Aspartic Acid treatment versus placebo in intercollegiate water polo players. Seven of the 9 WP subjects, or 77%, demonstrated greater power production with L-Aspartic Acid supplementation. Figure 3.4 illustrates the individual responses in power production ($W \cdot min^{-1}$) to L-Aspartic Acid treatment versus placebo in moderately-trained college males. Only four of the 9 MOD subjects, or 44%, demonstrated greater power production with L-Aspartic Acid supplementation.

Figure 3.5 illustrates the comparative percentage change in blood $NH_3$ [μM/L] and performance power ($W \cdot min^{-1}$) in WP and MOD in response to L-Aspartic Acid supplementation vs. placebo. Blood $NH_3$ [μM/L] dropped significantly ($F = 23.19$, $p < 0.0001$) with A (versus P) in WP by 16.7% ($241.0 \pm 18.4$ vs. $200.6 \pm 20.5$, $p < 0.01$) and in MOD by 15.5% ($219.7 \pm 20.8$ vs. $185.6 \pm 27.5$, $p < 0.05$). On the other hand, performance power ($W \cdot min^{-1}$) increased significantly ($p < 0.01$) with A (versus P) in WP by 22.7% ($270 \pm 19.7$ vs. $227.6 \pm 14.3$) with no significant change ($p > 0.05$) in MOD at 11.5% ($220.1 \pm 9.1$ vs. $204.4 \pm 8.3$). There was no significant difference ($p > 0.05$) in mean warm-up terminal wattage ($W \cdot min^{-1}$) between treatments (175 aspartate vs. 177 placebo).

Figure 3.6 illustrates individual changes (vs. placebo) in blood ammonia [μM/L] relative to the L-Aspartic Acid dose adjusted for body weight (mg/kg) in WP. As the relative L-Aspartic Acid dose (mg/kg) decreased in WP, to below 160 mg/kg, there was a tendency for blood ammonia concentration to drop (vs. placebo) by as much as [100 μM/L], which occurred at about 130 mg/kg. Figure 3.7 illustrates the individual changes (vs. placebo) in blood ammonia [μM/L] relative to the L-Aspartic Acid dose adjusted for body weight (mg/kg) in MOD. As observed in WP, blood ammonia [μM/L] tended to drop with lower dosages, however, with a higher degree of variability in MOD versus WP.

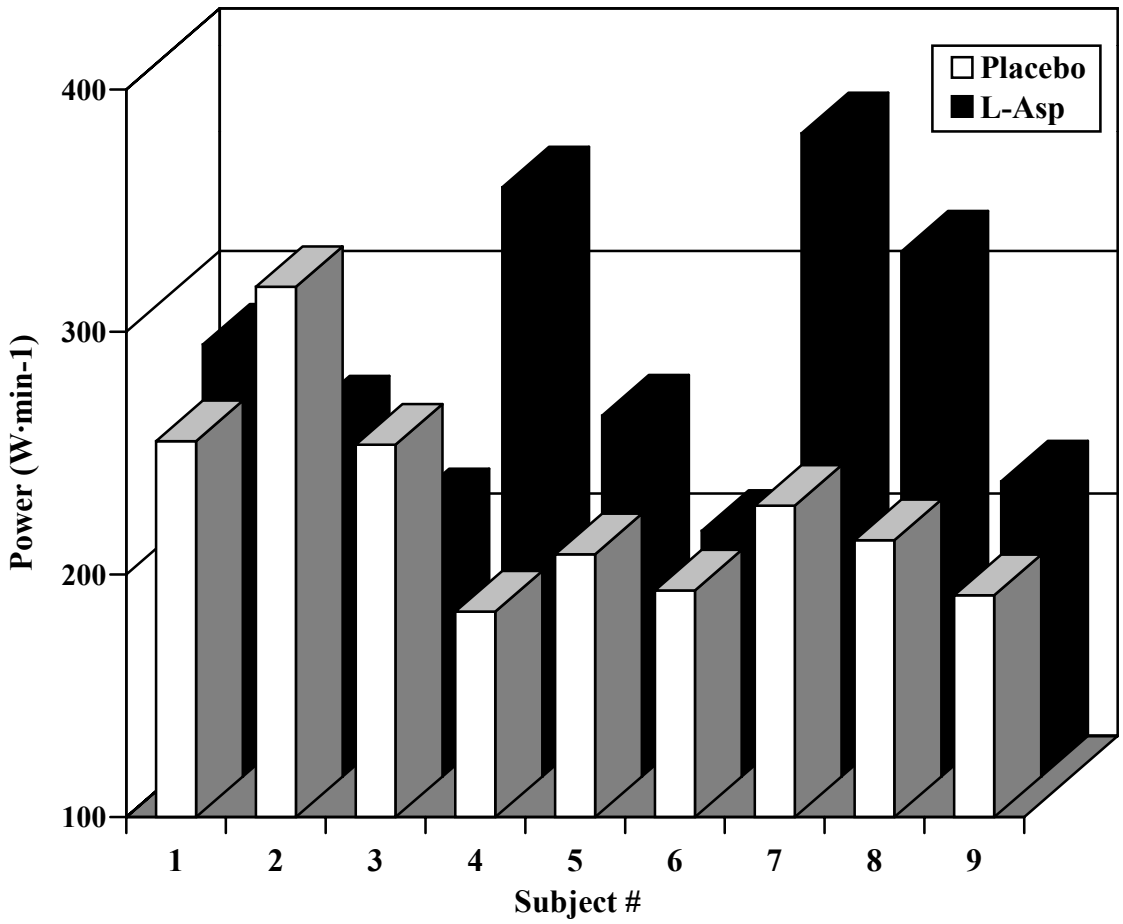


Figure 3.3. Individual responses in performance power ($W \cdot min^{-1}$) following treatment with either L-Aspartic Acid (L-Asp) or placebo in intercollegiate water polo players (n = 9).

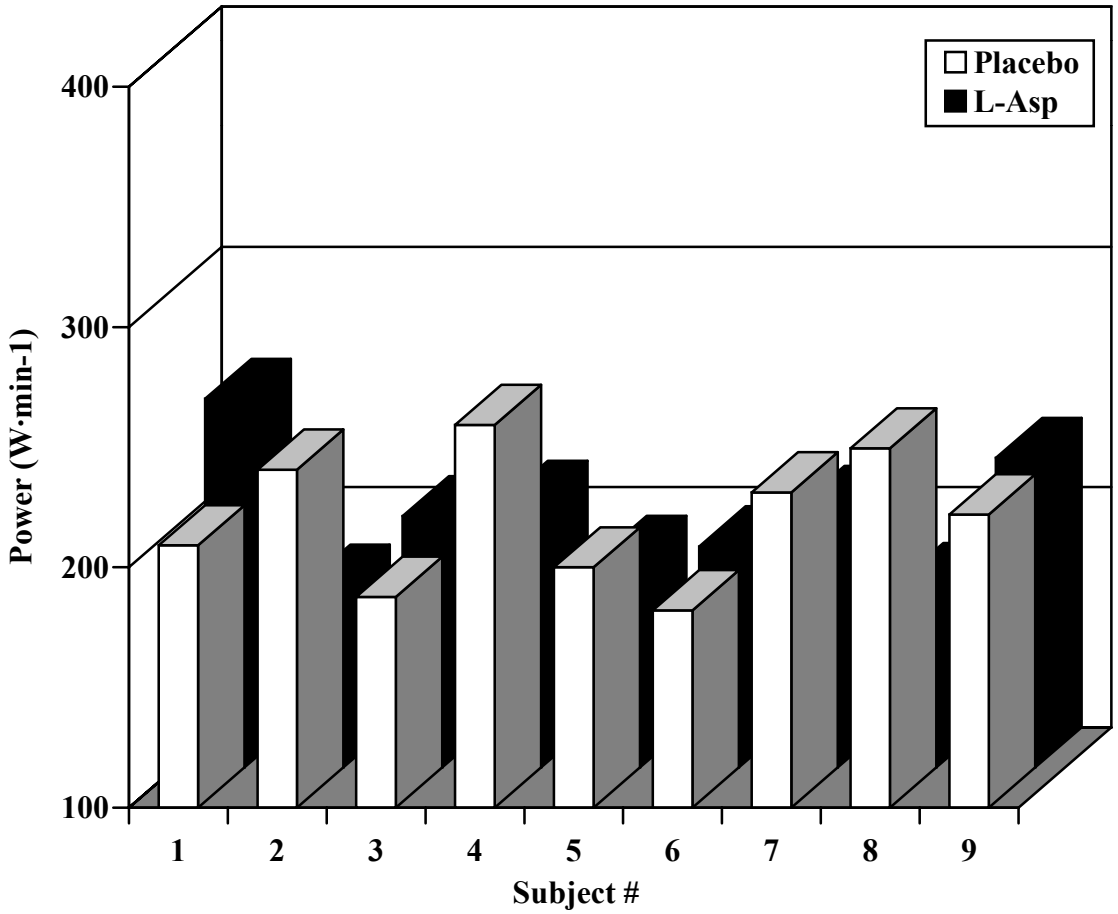


Figure 3.4. Individual responses in performance power ($W \cdot min^{-1}$) following treatment with either L-Aspartic Acid (L-Asp) or placebo in moderately-trained college-age males (n = 9).

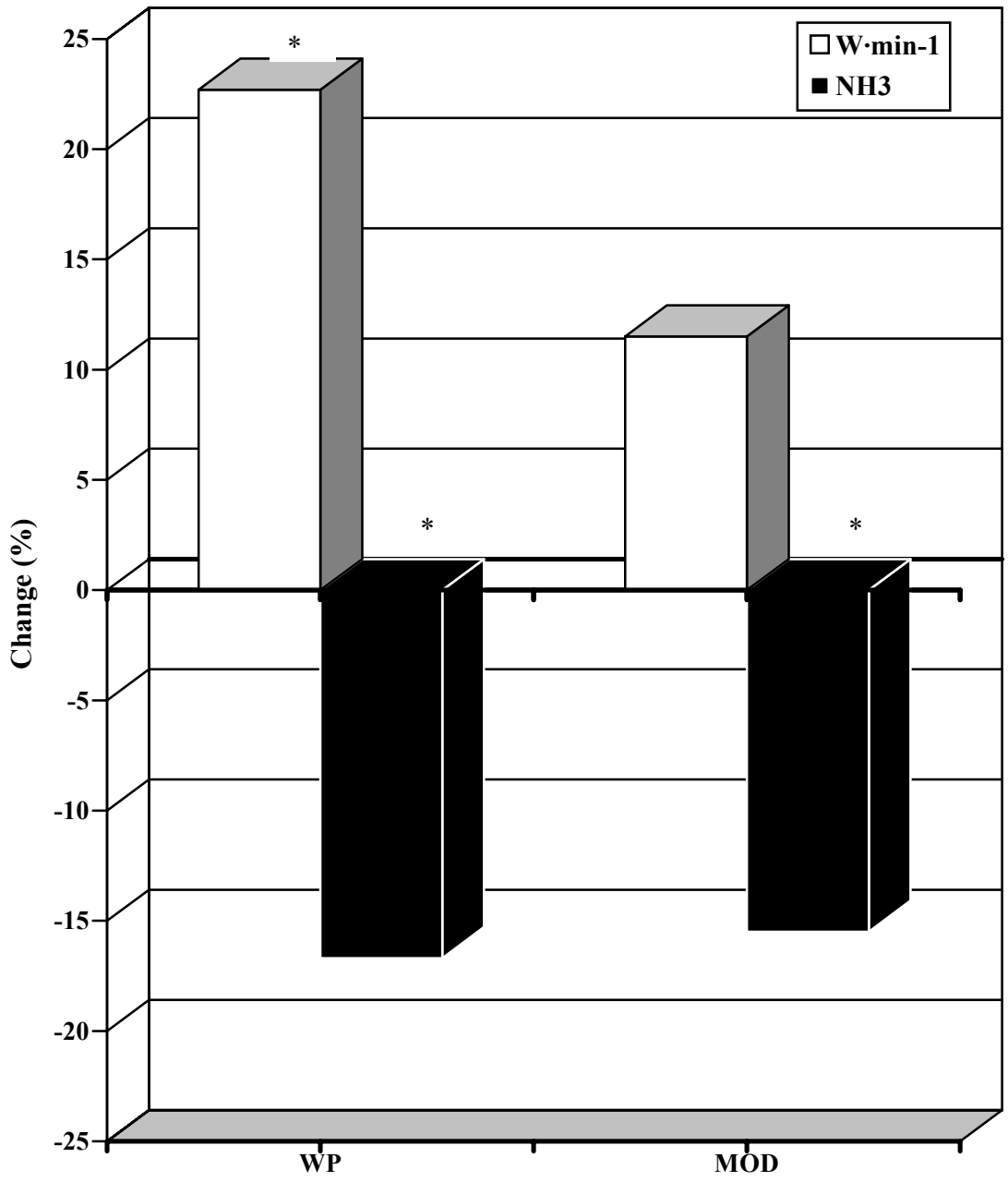


Figure 3.5. Mean percentage changes (vs. placebo) in blood $NH_3$ [μM/L] and performance power ($W \cdot min^{-1}$) in response to L-Aspartic Acid supplementation in water polo players (WP) and moderately-trained males (MOD).
*$p < 0.05$ versus placebo.

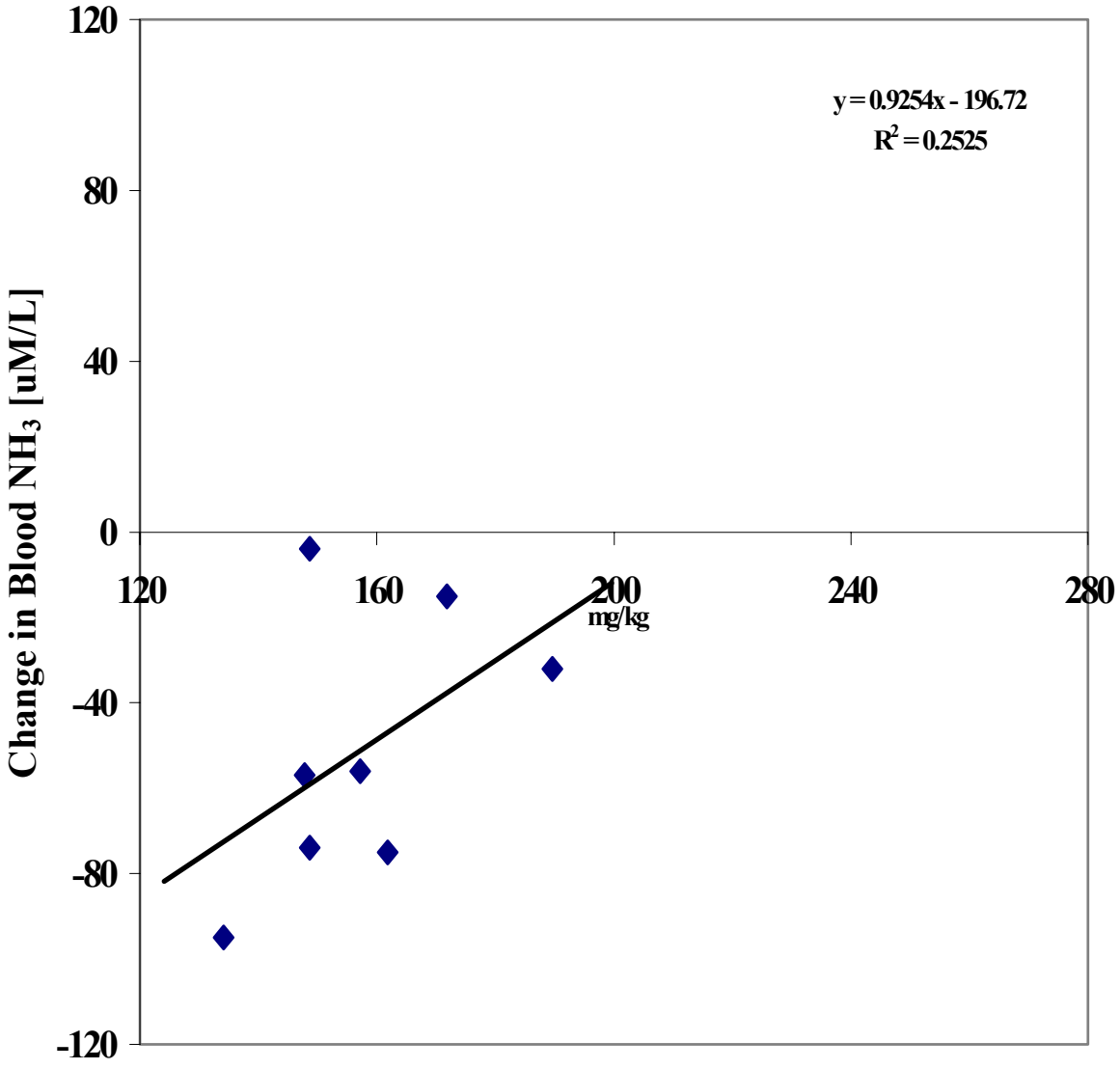


Figure 3.6. Individual changes (vs. placebo) in blood $NH_3$ [µM/L] relative to the L-Aspartic Acid dose adjusted for body weight (mg/kg) in intercollegiate water polo players.

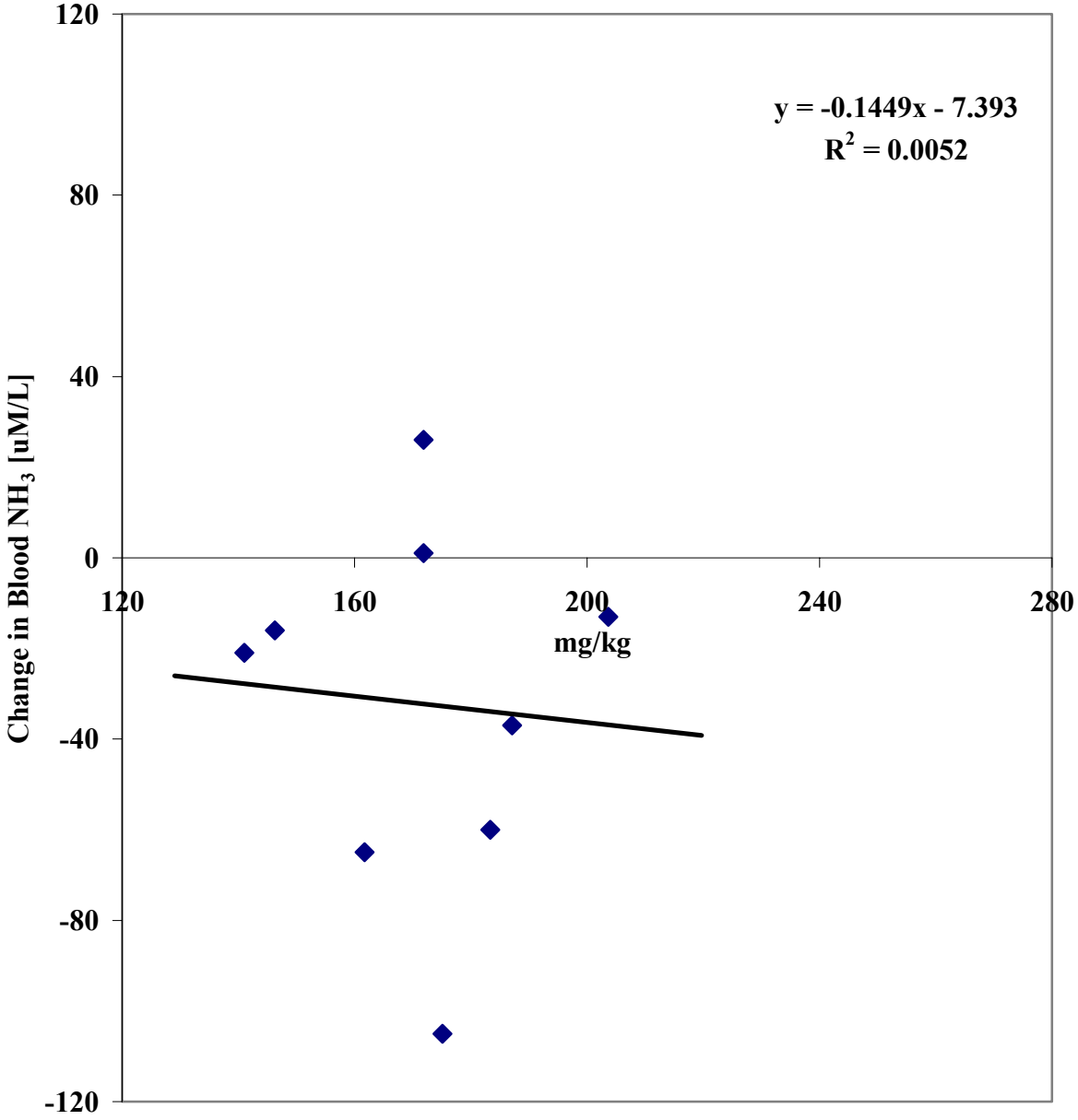


Figure 3.7. Individual changes (vs. placebo) in blood $NH_3$ [μM/L] relative to the L-Aspartic Acid dose adjusted for body weight (mg/kg) in moderately-trained males.

Figure 3.8 illustrates the individual changes (vs. placebo) in performance power ($W \cdot min^{-1}$) relative to the L-Aspartic Acid dose adjusted for body weight (mg/kg) in WP. There was a trend for a greater magnitude of performance enhancement (e.g. > 100 $W \cdot min^{-1}$ improvement) as the relative supplementation dosage was reduced, e.g., to below 150 mg/kg. Figure 3.9 illustrates the individual changes (vs. placebo) in performance power ($W \cdot min^{-1}$) relative to the L-Aspartic Acid dose adjusted for body weight (mg/kg) in MOD. This figure contrasts smaller (and fewer) improvements in power in MOD versus WP, with a higher (mean ± SD) relative dose per unit body weight (mg/kg) noted for MOD versus WP (171.3 ± 19.7 vs. 156.6 ± 16.3 mg/kg, respectively).

**3.4 Discussion**

This investigation tested the hypothesis that acute aspartic acid intake would 1) lower hyperammonemia and 2) improve high-intensity arm-crank power in two groups of college-age males. One group was highly-trained (i.e. in-season intercollegiate water polo players) for upper body activity, and the other was moderately-trained (i.e. weight training, surfing) for upper body activity. Both test groups included comparison to a placebo. We found a significant increase in short-term, high-intensity, upper body power output following L-Aspartic Acid supplementation in the highly-trained intercollegiate water polo players, but not in the moderately-trained group. Blood ammonia concentrations were lower with L-Aspartic Acid supplementation in both groups.

The current investigation found that 12.5 g x 2 QD of aspartate reduced exercise-induced hyperammonemia and enhanced short-term power in highly-trained male intercollegiate water polo players. On the other hand, acutely reducing $NH_3$ by aspartate supplementation did not conclusively improve short-term arm power in moderately-trained males. This suggests that hyperammonemia and performance response to aspartate supplementation are reflective of status and specificity of training. These improvements in

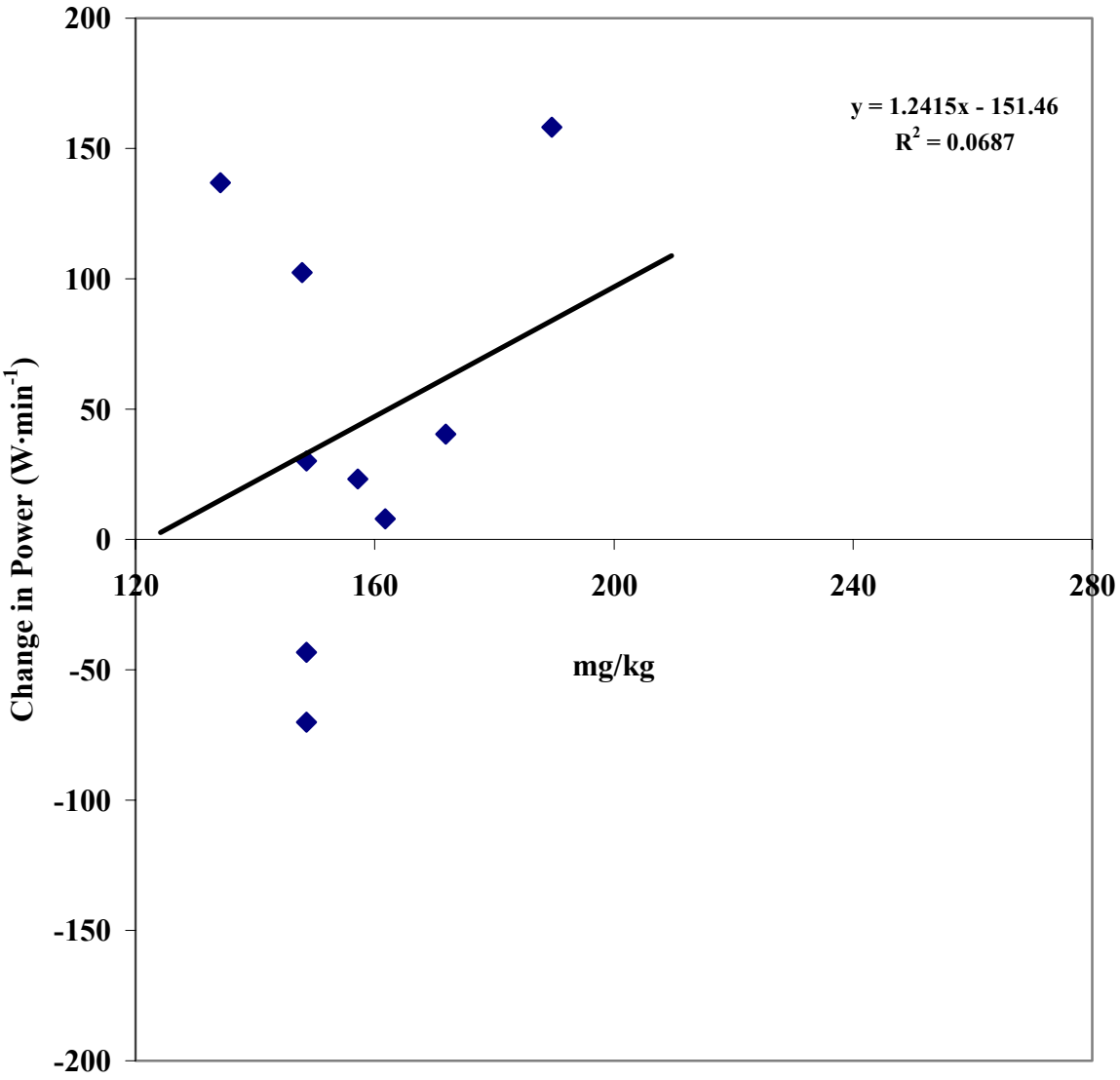


Figure 3.8. Individual changes (vs. placebo) in performance power ($W \cdot min^{-1}$) relative to the L-Aspartic Acid dose adjusted for body weight (mg/kg) in intercollegiate water polo players.

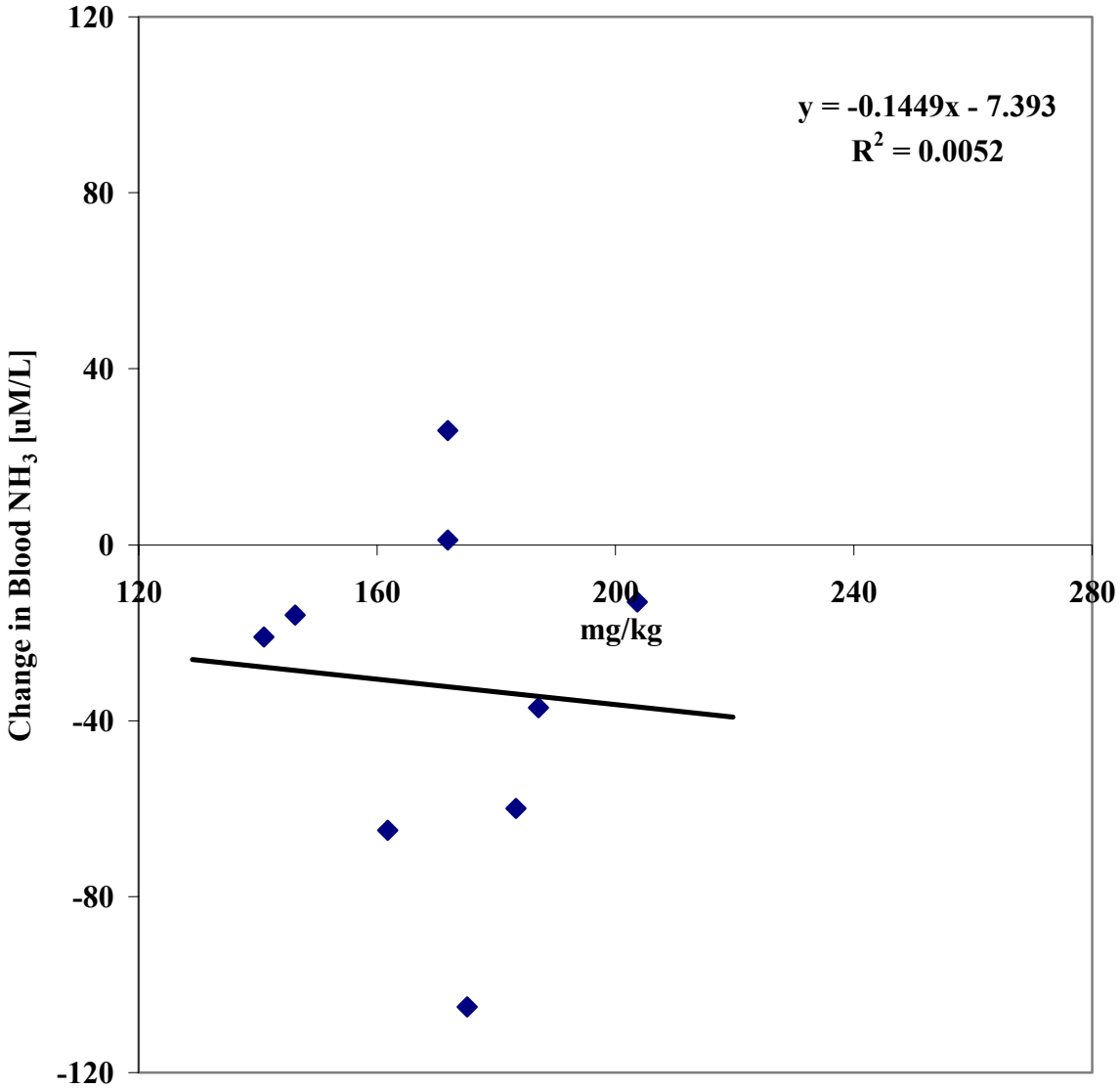


Figure 3.9. Individual changes (vs. placebo) in performance power ($W \cdot min^{-1}$) relative to the L-Aspartic Acid dose adjusted for body weight (mg/kg) in moderately-trained males.

trained subjects agree with the data of Wesson et al. (1988) and Ritter et al. (1998), and are at odds with Columbani et al. (2000). The small (i.e. ~10 - 20%) drop in hyperammonemia in the moderately-trained group may not have been great enough to create an observable increase in short-term power, which is in agreement with results with arm work by DeHaan et al. (1985) and Tuttle et al. (1995). However, these studies with upper body activity were of low to moderate intensity, which tend to keep blood ammonia levels under [100 μM/L] (Tuttle et al., 1995). In the current study, the L-Aspartic Acid dose (mean ± SD) expressed in terms of mg/kg body weight was 156.6 ± 16.3 versus 171.3 ± 19.7 in WP and MOD, respectively. The finding of the successful reduction of hyperammonemia coupled with lack of performance improvement in the lesser-trained group warrants further work in moderately-trained subjects to possibly consider different doses (e.g. 5, 7.5, 10g x 2 QD, relative to body mass) of L-Aspartic Acid.

Possibly, gluconeogenesis was activated to a greater extent in the moderately-trained group (versus well-trained) to meet ATP demand. This in turn would elevate the rate of production of alanine, pyruvate, lactate, OAA, glucose, ATP, and ammonia. The effects of aspartate supplementation on the production of each are unclear, however, as noted by Stryer (1995), accumulation of acetyl-CoA and pyruvate shifts bioenergetics toward OAA formation and gluconeogenesis, and thus away from the TCA cycle by the action of pyruvate carboxylase. Furthermore, studies conducted on animals show aspartate supplementation in untrained subjects (in singular and in combination) accelerates pyruvate carboxylase activity more than in well-trained individuals (Lancha et al., 1995, Lancha, 1997). For example, in response to high-intensity exercise, pyruvate carboxylase activity is higher in untrained (12x vs. rest) rat soleus versus trained (3x vs. rest). This suggests a glycogen sparing effect and less reliance on gluconeogenesis for ATP production with training, and a greater rate of gluconeogenesis for ATP production with reduced training.

However, as shown by Lancha et al. (1994), Lancha et al. (1995), and Lancha (1997), gluconeogenesis is accelerated at exhaustion. Therefore, one would also expect higher submaximal $NH_3$ values in lesser-trained subjects relative to power output, and more closely related ammonia values at exhaustion independent of training status. This expectation is consistent with observations made in this study.

It is possible that the ammonia removal response to aspartate supplementation was reduced in the moderately-trained subjects because the aspartate was used instead for gluconeogenesis. Aspartate is a substrate in both transamination (i.e. for gluconeogenesis) and in the urea cycle (for ammonia removal). During intense exercise, the bioenergetic flow of carbon and nitrogen changes to meet the primary demand for ATP production (i.e. gluconeogenesis). As a result, ammonia removal is compromised and hyperammonemia ensues. Previous work in our laboratory has found that isolating and enhancing urea production by supplementation with urea cycle amino acids (e.g. 10g each of L-arginine and L-Ornithine) did not improve high-intensity performance in moderately-trained subjects (Edwards and Day, 2000). In the present study, aspartate was more likely utilized for gluconeogenesis in the moderately-trained subjects (versus the well-trained) than for ammonia removal, which would account for the small reduction in ammonia across treatments and lack of power improvement. Therefore, there may be a threshold reduction in hyperammonemia (i.e. > 15%) required to create an observable performance improvement. These results warrant measurements of blood aspartate, oxaloacetate, aspartate aminotransferase, and pyruvate carboxylase to assess carbon and nitrogen bioenergetic flow with aspartate supplementation and high-intensity exercise.

An interesting sidelight from this research is the influence lactic acid has on high-intensity fatigue. Lactate levels and associated $H^+$ release are associated with fatigue (Hill et al., 1924). However, this dissertation found similar lactate levels for placebo and L-

Aspartic Acid supplementation, with greater performance in highly-trained subjects with L-Aspartic Acid supplementation. This suggests that blood [$NH_3$] (coupled with creatine phosphate depletion) may influence fatigue more than [blood lactate] in highly-trained individuals performing high-intensity arm work.

In summary, L-Aspartic Acid supplementation yields a significant increase in short-term, high-intensity, upper body power output in highly-trained intercollegiate water polo players, but not in moderately-trained subjects. While blood ammonia concentrations were lower with L-Aspartic Acid supplementation in both groups, the reduction in the moderately-trained group may reflect a larger portion of the aspartate diverted for gluconeogenesis. The metabolic ability of the higher trained subjects to remove blood ammonia and increase power in response to aspartate supplementation implies that hyperammonemia regulates fatigue more so than lactate in highly-trained subjects. For supplementation to be effective in moderately-trained populations, future work should focus on defining the existence of $NH_3$ reduction thresholds.

# CHAPTER 4. IMPACT OF L-ASPARTIC ACID SUPPLEMENTATION ON HIGH-INTENSITY EXERCISE HYPERAMMONEMIA AND PERFORMANCE IN FEMALES

## 4.1 Introduction

Strenuous physical activity (i.e. short-term intense work) elevates blood ammonia concentrations primarily by accelerating activity of the purine nucleotide cycle (i.e. AMP deaminase) and deamination of branched chain amino acids (Lowenstein, 1972, MacLean et al., 1996). As determined by Lo and Dudley (1987) and Levin et al. (1969), blood ammonia levels are inversely associated with exercise training status and performance, and rise during intense exercise to levels included in the diagnostic criteria (i.e. > [100 μM/L]) for clinical hyperammonemia (Batshaw, 1984). Symptoms of exacerbated clinical hyperammonemia (i.e. nausea, ataxia, dizziness) are likewise observed during intense exercise, where a normal resting blood [$NH_3$] of ~10-40 μM/L (Fischbach, 1988) rises to well over [100 μM/L].

Intake of the amino acid L-Aspartate is one of several amino acids (Najarian et al., 1958) that reduce the hyperammonemia associated with hepatic disorders, and, in some instances improves exercise performance in healthy populations (Ahlborg et al., 1968; Gupta et al., 1973; Wesson et al., 1988). Though controversial, a review of literature of the past 40 years indicates that an acute reduction in transient hyperammonemia in males by ~ 5 – 15 g QD of aspartate supplementation (Wesson et al., 1988, and Ritter et al., 1998) may enhance high-intensity exercise performance. However, the ability of L-Aspartic Acid supplementation to reduce exercise-induced hyperammonemia to enhance high-intensity exercise performance is unknown in females. Thus, the following experiment was conducted to address this concern.

## 4.2 Methods

### 4.2.1 Subjects

Eighteen recreationally upper body trained college-age females (FEM) attending Brigham Young University – Hawaii (BYU-H) volunteered for the study. Subject characteristics (mean, SEM, SD, minimum, and maximum) are presented in Table 4.1. Body weight (mean ± SD) (kg) was 61±7 (51-75). The study had the approval of the Institutional Review Board of the university, and subjects were informed of the nature of the study, the possible risks associated with participation, and of their right to withdraw at any time before providing written consent.

Subjects were instructed to abstain from alcoholic beverages, caffeine, and tobacco for the duration of the study, and, further, to keep dietary and exercise habits consistent across sessions. All subjects reported at least one year of experience of upper body recreational training 2-3x/week (i.e. weight training, surfing, swimming). None were members of an athletic team involving strenuous upper body training (e.g. swimming, water polo). Training status was characterized by the wattage ($W \cdot min^{-1}$) attained during the terminal stage of an incremental arm ergometer protocol at which an RPE of 17 (6-20 scale) was achieved.

Arm ergometry was chosen as the testing modality because it was specific to the upper body training status of the subjects. In addition, arm ergometry involved a smaller muscle mass, and therefore less endurance and less lab time. Further, testing via arm crank limited the confounding factors, which might have been observed due to inter-individual variations in day-to-day leg activity patterns.

### 4.2.2 Protocol

A counterbalanced double blind design determined the order of L-Aspartic Acid (A) or placebo (P) supplementation. A third party (office secretary) distributed two drink

powder packets to the subjects prior to each session. Subjects were instructed to mix the contents of each packet in 8 ounces of water, and ingest one drink 24 hours and the second

Table 4.1. Descriptive characteristics for recreationally-trained (FEM) females (n = 18).

| Subject Initials | Subject # (M/F) | Age (yr) | Height (cm) | Weight (kg) | Dosage (mg/kg) | $W \cdot min^{-1}$ @RPE 17 |
|---|---|---|---|---|---|---|
| AM | 1 | 21 | 157.5 | 52.7 | 237.1 | 100 |
| AH | 2 | 20 | 165.1 | 52.3 | 239.1 | 100 |
| CS | 3 | 22 | 167.6 | 57.3 | 218.3 | 125 |
| EP | 4 | 20 | 172.7 | 72.7 | 171.9 | 125 |
| EA | 5 | 20 | 177.8 | 61.4 | 203.7 | 100 |
| HH | 6 | 22 | 170.2 | 63.6 | 196.4 | 100 |
| JB | 7 | 20 | 167.6 | 51.4 | 243.4 | 100 |
| JS | 8 | 22 | 162.6 | 68.2 | 183.3 | 100 |
| KD | 9 | 22 | 165.1 | 47.7 | 261.9 | 100 |
| KW | 10 | 22 | 167.6 | 52.3 | 239.1 | 100 |
| LE | 11 | 25 | 180.3 | 75.0 | 166.7 | 100 |
| MR | 12 | 21 | 160.0 | 56.8 | 220.0 | 75 |
| NN | 13 | 20 | 167.6 | 59.1 | 211.5 | 75 |
| SC | 14 | 20 | 165.1 | 61.4 | 203.7 | 100 |
| SB | 15 | 21 | 167.6 | 59.1 | 211.5 | 100 |
| SM | 16 | 20 | 172.7 | 65.0 | 192.3 | 100 |
| TB | 17 | 19 | 167.6 | 63.6 | 196.4 | 100 |
| TF | 18 | 26 | 165.1 | 61.8 | 202.2 | 75 |
| Mean | | 21.3 | 167.8 | 60.1 | 211.0 | 98.6 |
| SEM | | 0.4 | 1.3 | 1.7 | 6.1 | 3.2 |
| SD | | 1.8 | 5.6 | 7.4 | 25.7 | 13.5 |
| Minimum | | 19.0 | 157.5 | 47.7 | 166.7 | 75.0 |
| Maximum | | 26.0 | 180.3 | 75.0 | 261.9 | 125.0 |

RPE - Rating of perceived exertion (6-20) scale
mg/kg – Relative dosage of 12.5 grams of L-Aspartic Acid per kg body weight

drink 1 hour prior to testing. One session followed ingestion of 12.5g x 2 of aspartate (Source Naturals, Inc., Scotts Valley, CA) mixed in 20g of powdered Powerade© (The Minute Maid Company for Coca-Cola USA, Northlake, IL), while the other session

followed ingestion of 20g x 2 of powdered Powerade© only. Powders were weighed to within 0.1g on a VWR Sargent Welch Calibrated Scale (VWR Scientific Products #WLS2648-30).

Subjects reported to the laboratory for two sessions of incremental seated arm ergometry (Monark Model #881). At least 48 hours separated each session. The exercise protocol consisted of two parts: 1) an incremental warm-up at a constant 100 rpm immediately followed by 2) all-out performance sprint to volitional exhaustion. The purpose of the warm-up was to have the subject achieve an RPE of 17 (very hard) to precede the sprint. The warm-up consisted of a series of 30-second stages, beginning with an initial intensity of 25W, and followed with an increase of 25W every 30 seconds until completion of the stage where an RPE of 17 was reached. Warm-up duration across sessions was the same intra-subject, while inter-subject the warm-up duration varied. Cadence (rpm) was monitored via electronic readout and the subject encouraged to maintain the correct cranking rate. RPM and total revolutions were monitored and tracked, respectively, via an integrated electronic readout. Verbal feedback was used to help subjects stay at target cadence during warm-up. The all-out power test to exhaustion began at completion of the warm-up stage where RPE reached 17 (6-20 scale). At that point, with a countdown of 5-4-3-2-1, and at a command of "GO!", the subjects turned the crank "as fast as possible for as long as possible". Warm-up revolutions, performance duration (seconds), and performance revolutions were recorded, and warm-up and performance power ($W \cdot min^{-1}$) were calculated via the formula:

$$W \cdot min^{-1} = (\text{standard ergometer wattage} / \text{standard revolutions}) \times (\text{revolutions} / \text{seconds}) \times (60 \text{ seconds} / 1 \text{ minute})$$

Seated blood samples were drawn 3 minutes post exercise. Following alcohol preparation of skin, a 21 gauge 1" Vacutainer© (Becton Dickinson and Co. Vacutainer Systems, Franklin Lakes, NJ) double-sided blood collection needle (#36-7212) was inserted into a superficial antecubital vein. A red top Vacutainer© tube with sterile interior was then inserted into the surrounding needle hub, and a 4-6 ml blood sample drawn for about 15 seconds. Blood samples were placed immediately into an ice bath for 10 minutes, and centrifuged for 10' at 3400 rpm (Fisher Scientific Centrific Model 228). Serum was transferred via disposable 3.2 ml polyethylene transfer pipettes (#13-711-7, Fisher Scientific, Pittsburgh, PA) into labeled 1.5 ml micro polypropylene metal-free test tubes (# 223-9480, Bio-Rad Laboratories, Hercules, CA), sealed with attached caps, and frozen immediately at 20 degrees Celsius for subsequent batch analysis. Serum [$NH_3$] was determined via a Kodak Ektachem Analyzer (Eastman Kodak Company, Rochester, NY) using Ektachem $NH_3$/Amon Clinical Chemistry Slides (Johnson and Johnson Clinical Diagnostics, Rochester, NY) using a phenol hypochlorite colorimetric technique.

#### 4.2.3 Statistical Analyses

Performance power ($W \cdot min^{-1}$) and serum [$NH_3$] were compared across treatments and groups using a repeated measures analysis of variance, with potential post-hoc differences analyzed using Tukey's t-tests. GB STAT statistical software was used for all analyses. Significance was set at $p < 0.05$.

### 4.3 Results

As specified per analyses, results are reported as the mean ± SEM, the actual value, the absolute change, or the percentage change (vs. placebo) in response to treatment. Figure 4.1 illustrates individual blood ammonia responses [µM/L] following either L-Aspartic Acid (L-Asp) or placebo in recreationally-trained college-age females. Nine of the eighteen subjects, or 50%, exhibited a reduction in blood ammonia concentrations with

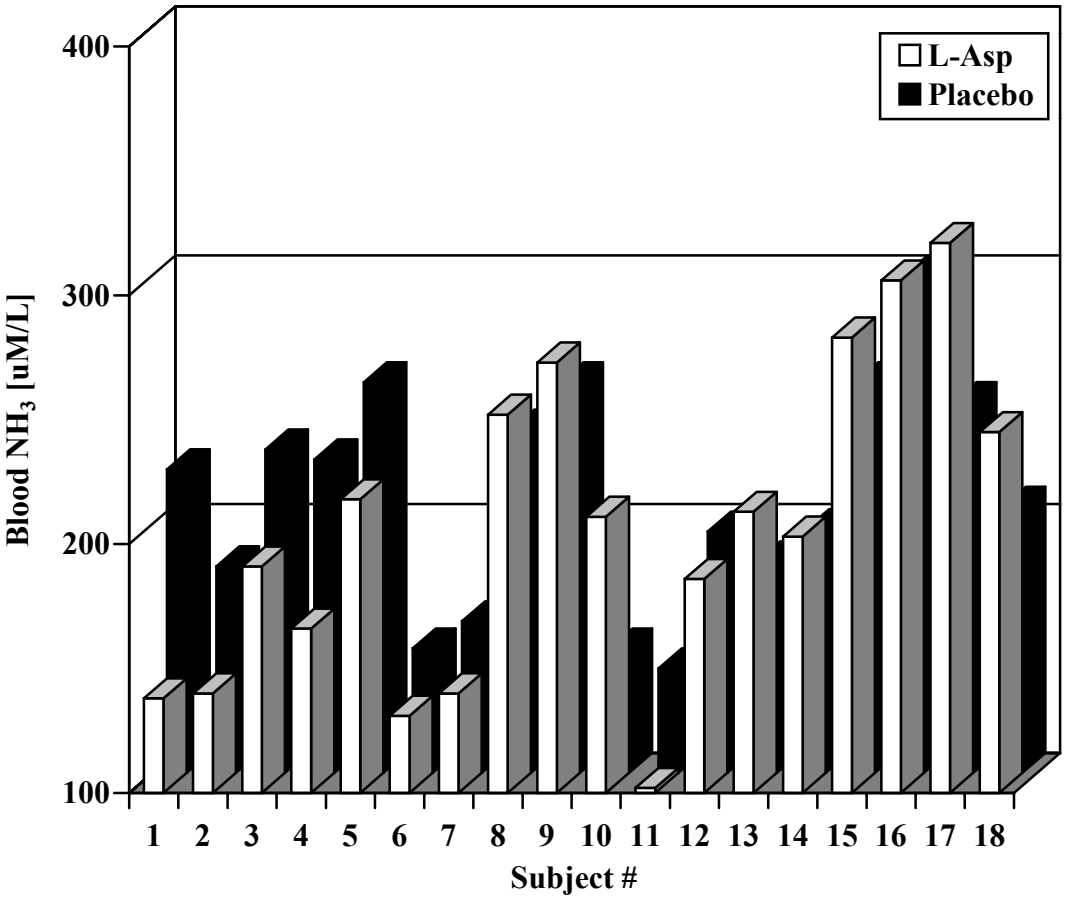


Figure 4.1. Individual responses in blood $NH_3$ [μM/L] following treatment with either L-Aspartic Acid (L-Asp) or placebo in recreationally-trained college-age females (n = 18).

supplementation, indicating a higher degree of variability in response with recreationally-trained college-age female subjects versus that observed in similar age males.

Figure 4.2 shows individual responses in performance power ($W \cdot min^{-1}$) following treatment with either L-Aspartic Acid (L-Asp) or placebo in recreationally-trained college-age females. Thirteen of the 18, or 72%, demonstrated greater power production with L-Aspartic Acid supplementation. However, the increases were relatively small when compared to the power increases observed in the male studies.

Figure 4.3 illustrates the comparative (%) change in power and $NH_3$ with L-Aspartic Acid supplementation versus placebo. There was no significant difference in (mean ± SEM) post-exercise blood [$NH_3$] between L-Aspartic Acid supplementation and placebo (204 ± 14 μM/L vs. 212 ± 10, respectively). Additionally, there was no significant change in performance power ($W \cdot min^{-1}$) between L-Aspartic Acid supplementation and placebo (172 ± 40 vs. 169 ± 36, respectively). Finally, no significant difference ($p > 0.05$) existed between treatments in warm-up power ($W \cdot min^{-1}$).

Figure 4.4 illustrates individual responses in absolute change (vs. placebo) in blood $NH_3$ [μM/L] relative to the L-Aspartic Acid dose adjusted for body weight (mg/kg) in recreationally-trained females. Further, Figure 4.5 illustrates individual responses in absolute change (vs. placebo) in performance power ($W \cdot min^{-1}$) relative to the L-Aspartic Acid dose adjusted for body weight (mg/kg) in recreationally-trained females. These illustrations reflect the apparent randomness in response of blood ammonia and performance power at the prescribed dose. In general, females have a higher relative (mg/kg)) dose versus males, thus, responses (or lack of) may be reflective of the relatively larger dose. Of note, in the current experiment the relative dose (mean ± SD) for FEM expressed in terms of mg/kg was 211.0 ± 25.7. At this average dose there was no trend for reduced hyperammonemia or performance improvement in recreationally-trained females.

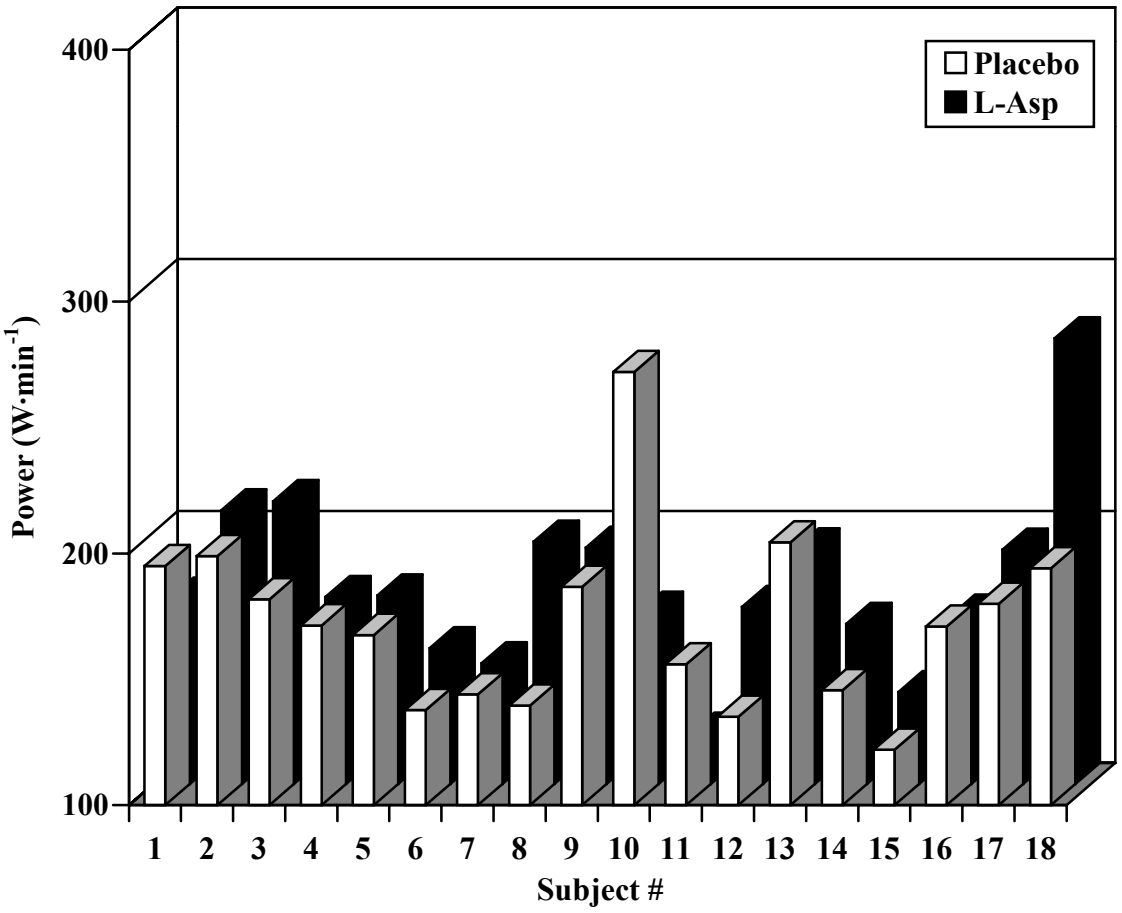


Figure 4.2. Individual responses in performance power ($W \cdot min^{-1}$) following treatment with either L-Aspartic Acid (L-Asp) or placebo in recreationally-trained college-age females (n = 18).

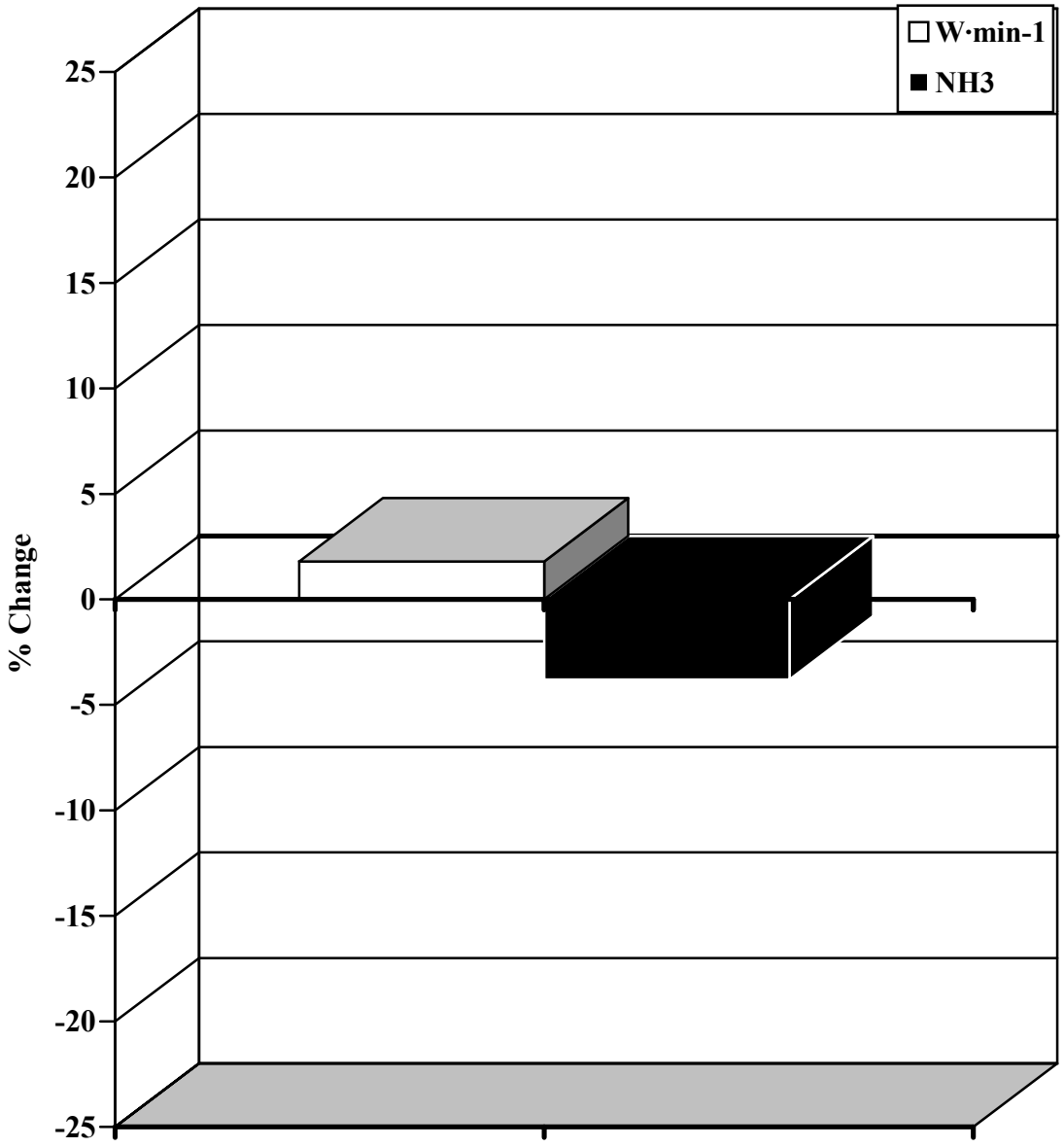


Figure 4.3. Mean percentage changes (vs. placebo) in blood $NH_3$ [μM/L] and performance power ($W \cdot min^{-1}$) in response to L-Aspartic Acid supplementation in recreationally-trained college-age females.

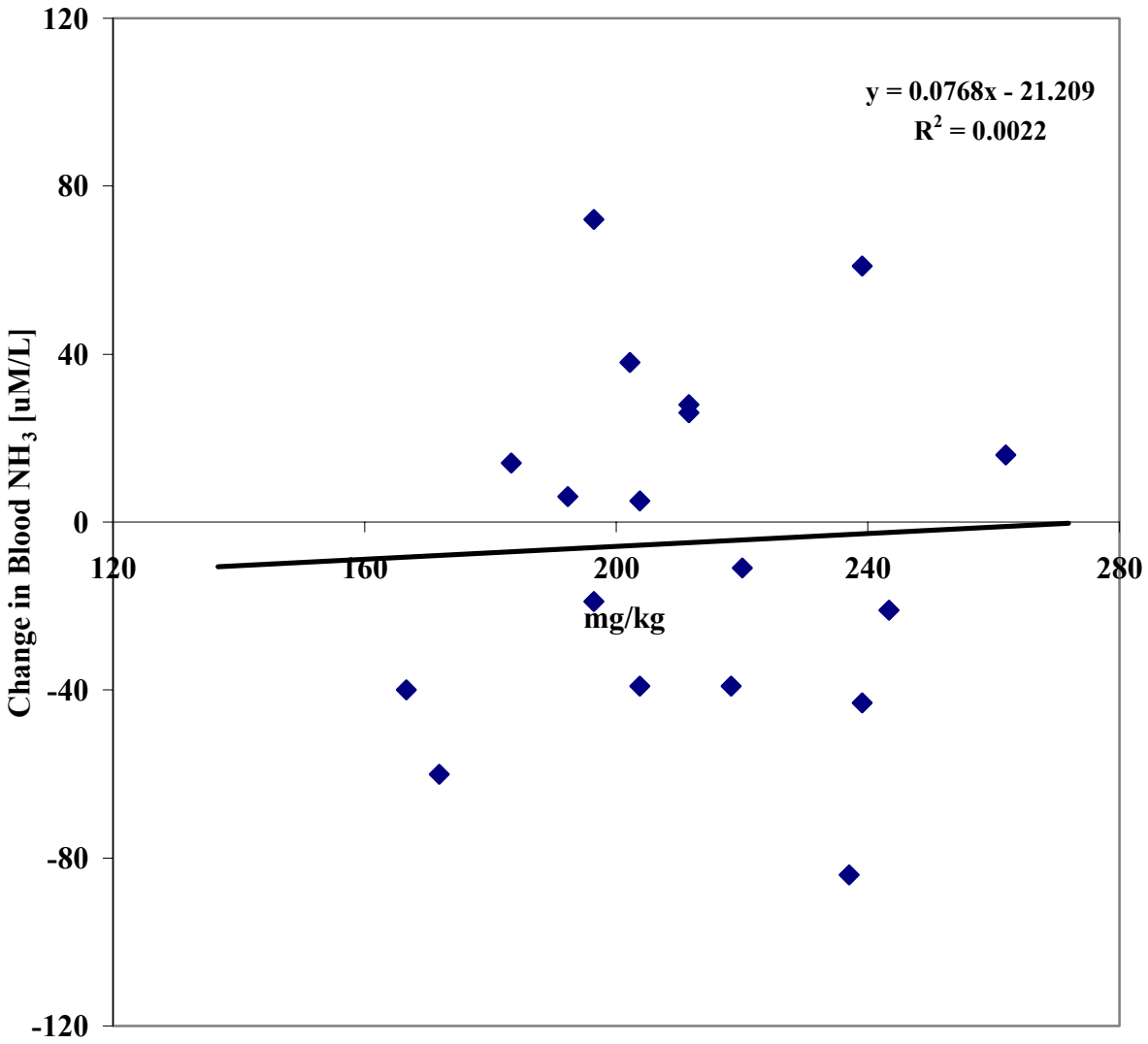


Figure 4.4. Individual changes in blood $NH_3$ [μM/L] relative to the L-Aspartic Acid dose adjusted for body weight (mg/kg) in recreationally-trained females.

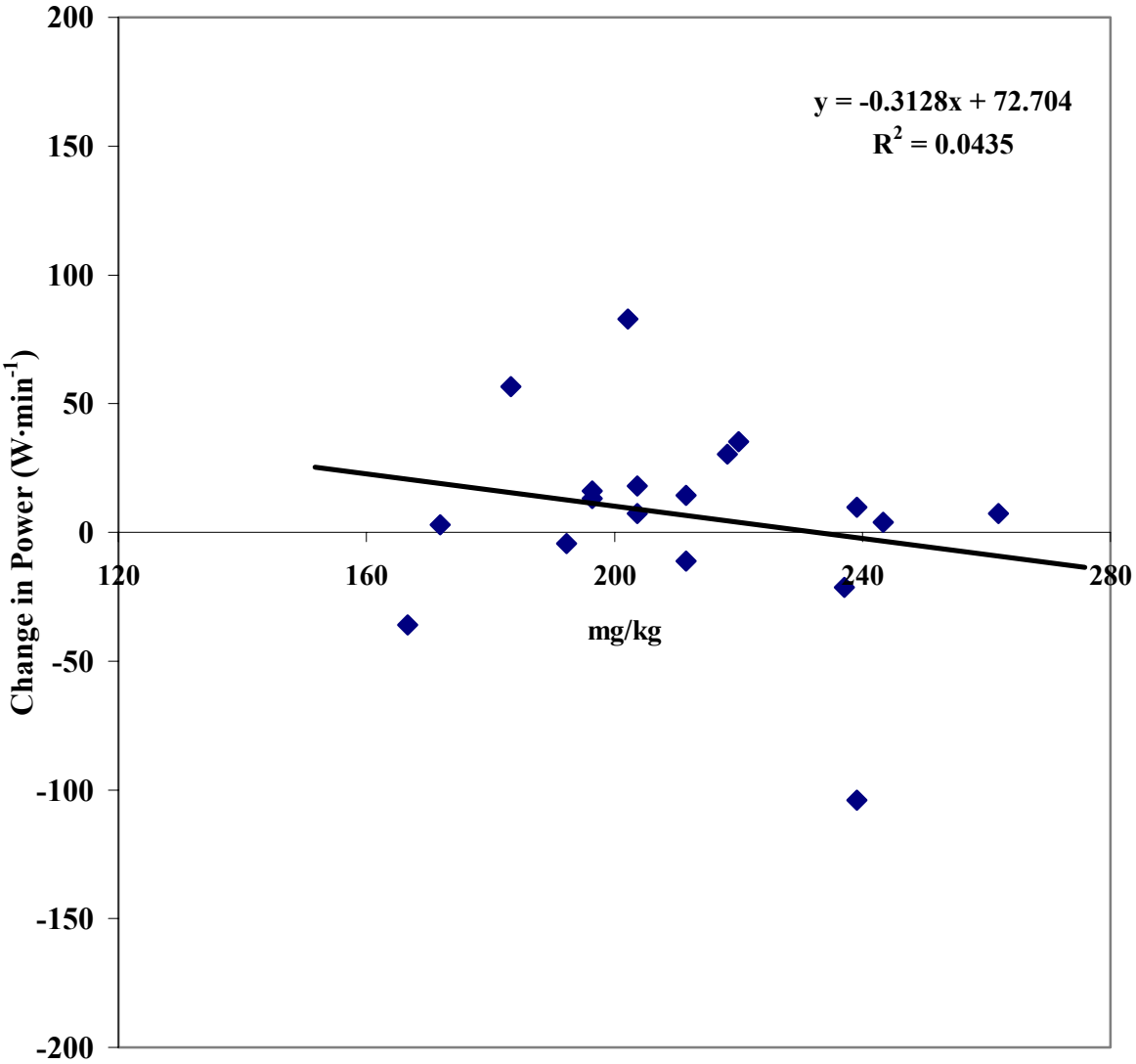


Figure 4.5. Individual changes (vs. placebo) in performance power ($W \cdot min^{-1}$) relative to the L-Aspartic Acid dose adjusted for body weight (mg/kg) in recreationally-trained females.

## 4.4 Discussion

This investigation is the first to report the effects of L-Aspartic Acid supplementation on exercise-induced hyperammonemia and high-intensity performance in females. We hypothesized that 12.5 g x 2 QD L-Aspartic Acid supplementation would increase short-term arm crank power and reduce associated hyperammonemia. However, hyperammonemia and performance power showed no significant response to treatment in recreationally-trained females. This is in agreement with the male studies of Hagan et al. (1982), Maughan and Sadler (1983), and De Haan et al. (1985), and is in contrast to the in studies in men that have shown performance improvement (Ahlborg et al., 1968; Franz and Chintanaseri, 1977; and Wesson et al., 1988), and the aforementioned investigation in this dissertation. The lack of response may be due to body mass, training status (recreationally-trained), testing modality, and/or training specificity. The possible interactions of these factors with aspartate supplementation are discussed below.

It is possible that the aspartate supplementation dose (12.5g x 2 QD, or ~ 205 mg/kg x 2 QD) in females was too high to the relative smaller body mass. While the mechanisms and regimen are unclear in humans, rat studies show an inverted dose response with hyperammonemia and exercise performance with aspartate supplementation (Laborit et al., 1958; Barnes et al., 1964; and Trudeau et al., 1993). Animal studies generally show a favorable response with doses between 10 mg/kg – 500 mg/kg QD (Laborit et al., 1958; Barnes et al., 1964; and Kendrick, 1976); doses over 500 mg/kg either increase performance (Rosen et al., 1962), have no effect (Matoush et al., 1964; Trudeau et al., 1993) or exacerbate hyperammonemia and worsen performance (Kendrick, 1976). Therefore, a lower dose of L-Aspartic Acid (i.e. 6g x 2 QD, or ~ 50 mg/kg - 100 mg/kg x 2 QD) in recreationally-trained females may generate a more desirable dose-response benefit.

Finally, since females generally have less absolute lean mass versus males, the magnitude of 'overdose' may have been even greater.

Training status (i.e. recreationally-trained) of the subjects may have also affected the lack of bioenergetic and performance response. Ammonia is a well-known factor in fatigue, with trained individuals removing ammonia more efficiently versus lesser-trained subjects (Graham et al., 1995a, Graham et al. 1997). Further, Lancha et al. (1995) demonstrated that pyruvate carboxylase activity is higher in untrained (12x vs. rest) rat soleus versus trained (3x vs. rest) with high-intensity exercise. Consequently, accelerated branched-chain amino acid catabolism for ATP production promotes higher exercising blood ammonia values. Additionally, Lancha (1997) found higher pyruvate carboxylase activity with aspartate and asparagine supplementation in untrained rats at all times in response to high-intensity exercise (versus control). This finding however did not occur in trained rats. Therefore, the L-Aspartic Acid supplement in the present study may have been recruited more as a substrate for gluconeogenesis instead of for ammonia removal. This in turn would 1) produce greater ammonia per unit of ATP, 2) reduce a major source of the ammonia removal mechanism, and 3) make less aspartate available as a substrate for ammonia removal in the urea cycle. Therefore, the net result would be unchanged blood ammonia values (or slightly higher than anticipated) and lack of performance improvement.

Additionally, the lack of specificity of the training of the subjects to the testing modality may have failed to affect the bioenergetic (and thus performance) response to aspartate supplementation. As mentioned previously, Lancha et al. (1995), showed that in response to high-intensity exercise, pyruvate carboxylase activity is higher in untrained rat soleus versus trained. Secondly, Lancha et al. (1994) demonstrated in both trained and untrained rats supplemented with aspartate and asparagine, a three-fold greater conversion

(vs. placebo) of acetyl-CoA and OAA into citrate and coenzyme-A during exhaustive exercise. Third, Lancha et al. (1995) demonstrated increased plasma aspartate, glycogen content, FFA's, and prolonged (+42%) swim time to exhaustion with supplementation of aspartate, asparagine, and carnitine. Therefore, these data suggest that the bioenergetic response to aspartate supplementation is reflective of training status, promoting a much larger activity of both gluconeogenesis and the oxidative systems. On the other hand, if one assumes recreationally-trained subjects have a reduced capacity of (or receptiveness to) the pathways affected by aspartate, then a greater (or lower) availability of aspartate may have no effect at all on ATP production or ammonia removal. In other words, if high-intensity exercise pathways are either not present (in greater amounts) or are unresponsive to aspartate supplementation, then no outward effect will be observed.

Therefore, these results suggest that response to L-Aspartic Acid supplementation in recreationally-trained females may be dependent on both body mass and training status, either singularly or in combination. In summary, the present investigation found no effect of L-Aspartic Acid supplementation on short-term power or hyperammonemia in recreationally-trained females. For supplementation to be effective, future work may consider 1) a more highly-trained group of females, 2) a dose-response study in proportional to the lower body mass in females (i.e. 0 mg/kg, 50 mg/kg, 100 mg/kg, 150 mg/kg ), and /or 3) a protocol more specific to the metabolic status of the subject group.

# CHAPTER 5. SUMMARY, QUESTIONS AND ANSWERS, AND CONCLUSION

## 5.1 Summary

This series of investigations is the first to report the effects of L-Aspartic Acid supplementation on ammonia reduction and high-intensity arm-power. We found a significant increase in short-term, high-intensity, upper body power output following L-Aspartic Acid supplementation in highly-trained intercollegiate water polo players, but not in moderately-trained or recreationally-trained subjects. Previous reports in the literature have concentrated on medium to long duration time to exhaustion. This dissertation is also the first series of studies to investigate the dose-response effect of L-Aspartate ingestion and blood ammonia, and assess the reliability of the ammonia assay across sessions. Results from this dissertation determined the ammonia assay to be reliable, validating the assessment of blood ammonia concentration in response to aspartate supplementation and high-intensity exercise.

Pilot work provided general trends as to the metabolic (glycolytic and nitrogen) and performance effects of various combinations of ammonia reducing amino acids. Pilot results suggest that blood urea elevation exclusively does not deter, nor promote exercise-induced hyperammonemia nor increase exercise performance. Secondly, of the ammonia reducing amino acids (i.e. glutamate, arginine, ornithine, and aspartate), L-aspartate appears to be the most effective for reducing short-term exercise-induced hyperammonemia, if ingested in the 5 - 15g x 2 range over the 24 hours preceding exercise. Furthermore, a carryover/storage effect with aspartate ingestion enhances blood ammonia reduction across consecutive days of high-intensity exercise. Although previous work has shown that aspartate supplementation over the week preceding long duration exercise (e.g.. a marathon) is not beneficial, the performance effect of high-intensity exercise in response

to L-Aspartic Acid storage is unknown. Results from this dissertation suggest that high-intensity short-term power and blood ammonia response to aspartate supplementation are training status dependent. This is in contrast with the hypothesis of a general lowering of exercise-induced hyperammonemia with L-Aspartic Acid supplementation and improvement in performance independent of training status. The effect on exercise-induced hyperammonemia by L-Aspartic Acid supplementation appears to be proportional to training status (i.e. the highly-trained water polo group had the largest drop, followed by the moderately-trained males, with no effect on blood [$NH_3$] in recreationally-trained females). In other words, the more highly-trained subjects responded more favorably in both reduction of hyperammonemia and improvement in performance with aspartate supplementation, while the lesser trained groups did not. This drop in blood ammonia was associated with increased short-term power output in highly-trained intercollegiate water polo players. Application of these results would include athletic events where small increases in power may be desirable, i.e. water-polo sprinting.

The absolute aspartate (or placebo) dose regime per session per subject was identical across the three investigations in the dissertation. Due to greater overall body weight, males therefore have a lower (mean ± SD) relative lower dosage (mg/kg) of 12.5 g of L-Aspartic Acid (156.6 ± 16.3, and 171 ± 19.7, WP and MOD, respectively) than the females (211.0 ± 25.7). Therefore, as illustrated in Figure(s) 5.1 and 5.2, it is conceivable that per unit of body weight (mg/kg) the 12.5 g dosage in the recreationally-trained females was too high to elicit favorable responses in either hyperammonemia or performance power. Although human results are inconclusive, data in animal models (Matoush et al., 1964; Kendrick, 1976; and Trudeau et al., 1993) suggest an inverted dose-response effect (lack of ammonia reduction and/or performance improvement) as dosages exceed a certain level (i.e. in animals, approximately 500 mg/kg, independent of training status or gender).

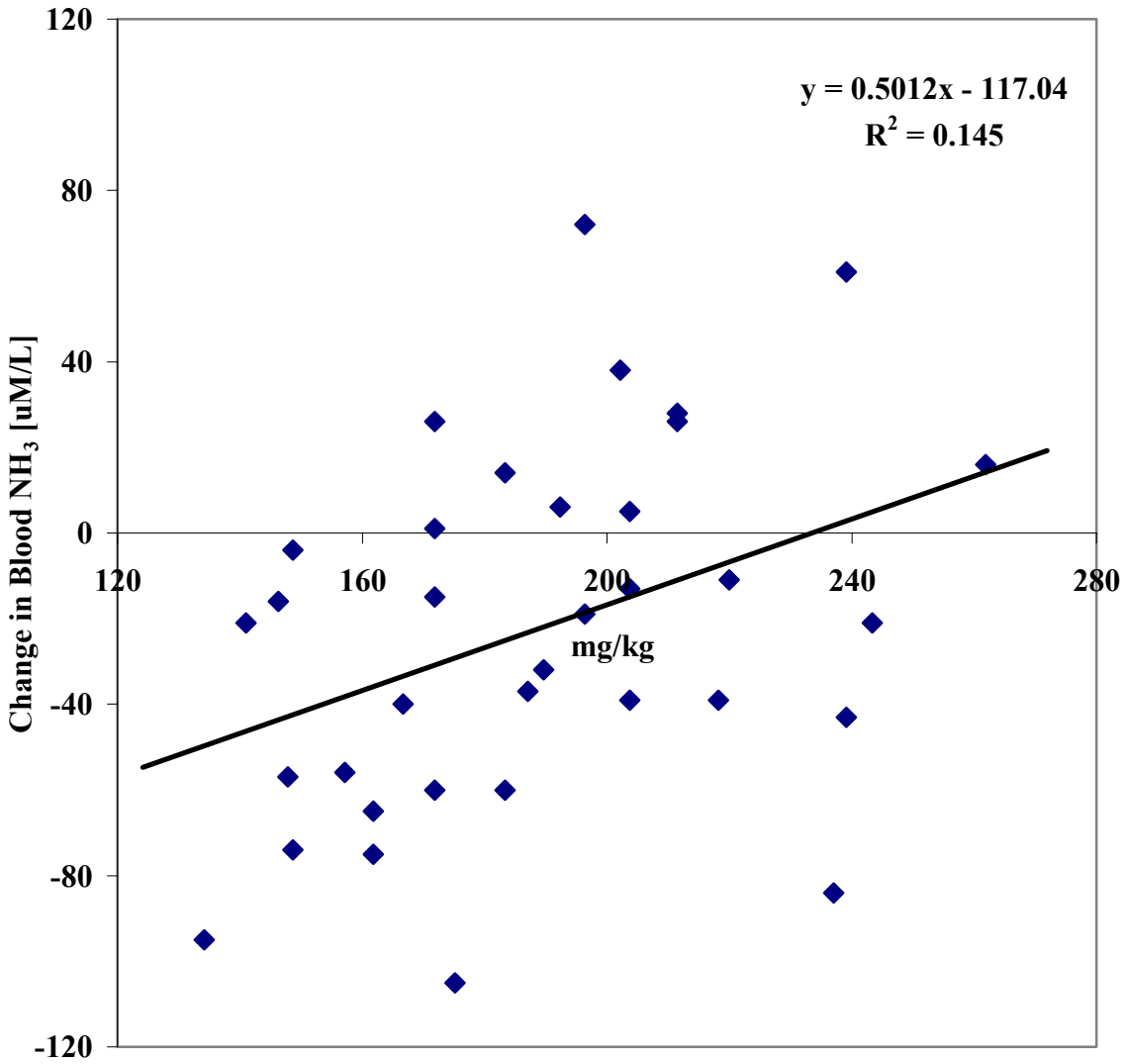


Figure 5.1. Individual changes (vs. placebo) in blood ammonia [μM/L] relative to the L-Aspartic Acid dose adjusted for body weight (mg/kg) in males and females.

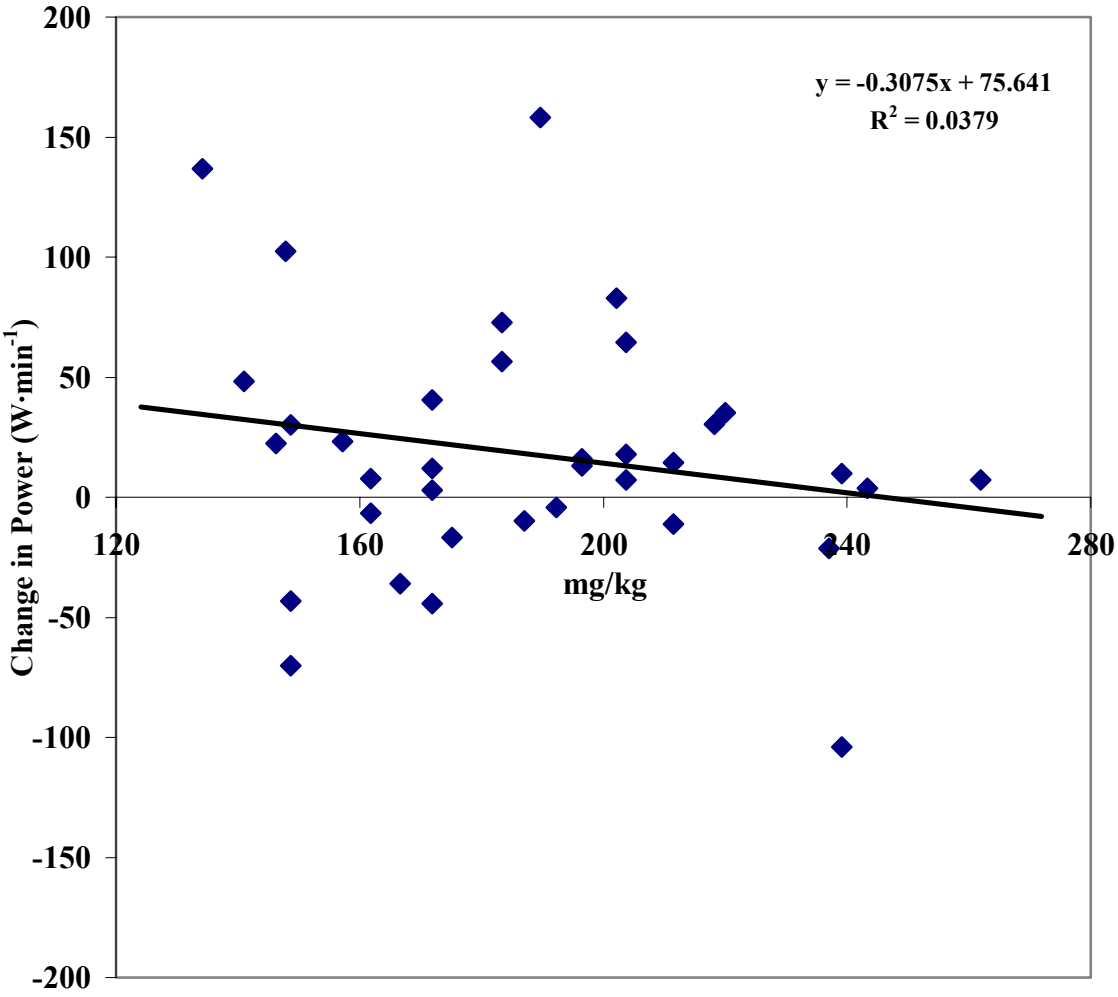


Figure 5.2. Individual changes (vs. placebo) in performance power ($W \cdot min^{-1}$) relative to the L-Aspartic Acid dose adjusted for body weight (mg/kg) in males and females.

Results from the dissertation suggest this to be relative doses exceeding 175 mg/kg in college-age males and females, independent of training.

The training status of the subjects may also have affected the metabolic response to aspartate supplementation. Previous work by Neame and Wiseman (1957) shows that blood levels of aspartate peak in the circulation between 20 and 40 minutes following introduction to the intestinal lumen, with concurrent mesenteric alanine blood levels rising almost 2-fold. Alanine is a major source of both bioenergetic carbon and nitrogen, which thus affects blood ammonia concentration. Further, it is possible the relative lack of training in the oxidative systems in the lesser trained subjects, and increased activity of gluconeogenesis diverted aspartate away from the TCA cycle and the urea cycle. This would explain the lack of an observable performance improvement or enhanced reduction of hyperammonemia with lack of training.

This dissertation is also the first to report the impact of L-Aspartic Acid supplementation on blood ammonia concentration and high-intensity power response in females exclusively. Figure(s) 5.1 and 5.2 illustrate the general favorable trend with a reduction in hyperammonemia and increase in power with a relatively lower L-Aspartic Acid supplementation dose expressed in terms of body weight (mg/kg). However, these results indicate no treatment effect on either blood ammonia concentration or high-intensity power in recreationally-trained females with 12.5g x 2 QD of L-Aspartic Acid supplementation. However, the females did not exhibit reduced hyperammonemia or an increase in power as observed in the more highly-trained men. The possibility exists that the females were overdosed per unit of body weight, thus warranting more dosage titration work relative to total body mass (and lean mass as well). Furthermore, more work is needed in females who are more specifically trained to the testing modality (e.g. cross-

country runners, treadmill running, female surfers) to therefore rule out the possible effect of gender.

The type of protocol may have affected the toleration of exercise above the anaerobic threshold in the lesser trained groups. In addition, the lack of metabolic system response to aspartate supplementation in the lesser trained may also be due to lack of specific training to the arm crank movement. The metabolic ability of the higher trained subjects to remove blood ammonia and increase power in response to aspartate supplementation, implies that hyperammonemia regulates fatigue more so than lactate in highly-trained subjects.

Therefore, it appears that both ammonia clearance and aspartate bioenergetic metabolism (i.e. aspartate substrate, alanine, fumarate, OAA, adenine nucleotide production) affect power production in response to L-Aspartic Acid supplementation. Results from this dissertation suggest the impact is regulated by training status, relative aspartate dosage, and specificity of training. Athletes participating in sports as competitive swimming, water polo sprinting, and wheelchair racing may benefit from supplementation. Although supplementation appears effective in well-trained subjects, the suggested amount of supplementation relative to body weight (mg/kg) and status of training is unclear, and warrants further work.

In summary, these results suggest that aspartate ingestion may be an effective nutritional tool for highly-trained athletes in competitions requiring short-term power bursts (e.g. water polo sprinting), where a small improvement in power may be desirable. Caution should be exercised for applying a crossover effect into the other competitive athletic modalities, and for applications involving active recruitment of lesser-trained muscle groups of the trained populations.

## 5.2 Questions and Answers

### 5.2.1 Questions Answered

The following questions were answered by this dissertation:

1) Does aspartate supplementation reduce blood [$NH_3$] and improve high-intensity short-term power?

Answer: In some cases. Yes in the well-trained test group. No for the recreationally-trained group. No for the moderately-trained group although small drops in $NH_3$ levels were observed.

2) Is there a dose-response effect between variable ingestion (0,5,10,15g) of aspartate and high-intensity blood [$NH_3$]?

Answer: Yes and No. A general lowering of hyperammonemia is observed with 5, 10, or 15 g of aspartate versus placebo. However, it appears there is no difference in response between 10 and 15 grams. Status and specificity of training affect the magnitude of response.

3) Is there an inverse relationship between maximal performance indices and blood [$NH_3$] at exhaustion, with state of training?

Answer: The relationship is unclear, but doubtful. Present work showed similar $NH_3$ levels (~ [200 – 250 μM/L]) across training status at exhaustion independent of that status - but higher power output with trained subjects.

4) Is there an 'ammonia tolerance' threshold, i.e. an absolute blood concentration or percentage change from rest, where the subject requests to stop?

Answer: Probably. Ammonia tolerance seems to depend on training status, task familiarity, and intensity familiarity. With short-term protocols, blood [$NH_3$] between 200 and 250 μM/L are tolerated with difficulty; with $NH_3$ > [250 μM/L] the subject requests to stop.

5) Is ammonia tolerance and power output during high-intensity exercise more reflective of status of specificity of training?

Answer: Yes. The highly-trained test group were able to generate more power than the moderately-trained test group at similar $NH_3$ concentrations and further, respond favorably to aspartate supplementation reduce blood ammonia, and increase power even more.

6) Is there a crossover effect of blood ammonia clearance, in regard to training status across testing modalities (arm ergometry testing vs. leg-trained subjects)?

Answer: Not observed in the present study, marginal improvement at best.

7) Were the levels of $NH_3$ at exhaustion lower in women versus men?

Answer: Yes and No. In absolute terms, $NH_3$ levels for the women in this dissertation were comparable to males. Relative to power output, $NH_3$ levels for women were higher; however, the effect of gender is unknown, suggesting future work in a higher-trained group of females well-trained to the testing modality.

8) How much blood ammonia reduction (%, [μM/L]) is required to observe changes in high-intensity power?

Answer: Depends on training status. In the highly-trained test group $NH_3$ reductions of least 15% yielded an observable increase in power output. In the other tests groups, supplementation did not result in appreciable decreases in $NH_3$; warranting future dose-response work.

### 5.2.2 Future Questions to be Investigated

1) Is there a dose-response effect between variable ingestion (0,5,10,15g) of aspartate, and maximal short-term performance power?

Answer: Unknown

2) Do females have a different ammonia tolerance capacity versus males?

Answer: Unclear. To address this, future work may consider 1) a more highly-trained group of females, 2) a dose-response study in proportional to the lower body mass in females (i.e. 0 mg/kg, 50 mg/kg, 100 mg/kg, 150 mg/kg), and /or 3) a protocol more specific to subject metabolic status.

3) Do subjects have a crossover 'ammonia tolerance' threshold(s) of specificity of training, for arms vs. legs?

Answer: Doubtful, marginal at best

## 5.3 Conclusion

It appears from this dissertation that 12.5 g x 2 QD of aspartate reduces hyperammonemia and enhances short-term power in intercollegiate water polo players. However, in moderately-trained males or recreationally-trained females, 12.5 g x 2 QD of aspartate does not attenuate hyperammonemia to the degree to improve power.

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## APPENDIX A. SUBJECT CONSENT

The Impact of Aspartate Ingestion and Ammonia Reduction on Exercise Metabolism and Performance (Randy Day, Ph.D., William W. Edwards, M.S.)

In order to investigate the effects of amino acid ingestion on upper body performance, I hereby volunteer to participate in an experiment conducted by the Dept. Of Biology of BYU-Hawaii. I have been informed of the nature of the experiment, and of the risks involved in an exercise test. I understand that an amino acid supplement will be mixed in a commercial performance beverage for consumption prior to testing, and agree to keep my dietary and exercise habits consistent across testing days, and to abstain from caffeine, alcohol, and tobacco use for the duration of the experiment.

I acknowledge that a venous forearm blood draw will be performed following each exercise test by trained personnel. The data obtained from my participation will receive impersonal statistical treatment, with my rights to privacy protected. My personal identity remains anonymous unless permission is granted otherwise.

Furthermore, I may withdraw from the experiment at any time. Any questions concerning the experiment have been answered to my satisfaction, and I understand that I may ask further questions once the experiment is complete.

_____
Name (printed)

_____ _____
Signature Date

_____ _____
Witness Date

# APPENDIX B. PILOT STUDY 1: THE EFFECTS OF SUPPLEMENTATION OF AMMONIA REDUCING AMINO ACIDS ON EXERCISE METABOLISM AND HIGH-INTENSITY PERFORMANCE

## B.1 Introduction

Previous work has suggested that an acute reduction in blood ammonia by ingestion of ammonia reducing amino acids may improve medium and long duration athletic performance. However, it is unclear as to whether the reduction in blood ammonia in this manner may improve short-term high-intensity performance (endurance, strength, power, speed) where blood ammonia values peak. Therefore, this study explored the effect of ingestion of a combination of ammonia-reducing amino acids on blood ammonia, urea, lactate, and exhaustion time in response to high-intensity cycling ergometry.

## B.2 Methods

### B.2.1 Subjects

Three moderately-trained college-age males were informed of the nature of the study, and of the possible risks involved, before giving written consent in accordance with university guidelines and approval by the institutional review board of Louisiana State University, Baton Rouge, LA. Descriptive statistics are presented in Table B.1.

### B.2.2 Protocol

All exercise tests were performed on a calibrated friction-braked bicycle ergometer (Monark #860) at a pedal frequency of 80 rpm. Each subject performed a preliminary incremental exercise test for assessment of $VO_{2max}$. Following instrument calibrations and a 2-minute exercise warm-up, the testing began at a resistance of 50 watts, and increased 50 watts/minute until metronome cadence dropped to lower than 60 rpm with best effort.

Oxygen consumption, carbon dioxide production ($VCO_2$), and expired ventilation ($V_E$) were measured via open-circuit spirometry. Normoxic inspired gas volumes were monitored by a dry gas meter. Subjects breathed through a low-resistance (Rudolph)

Table B.1. Descriptive characteristics of subjects in pilot study 1.

| Variable | Subject 1 (MC) | Subject 2 (RM) | Subject 3 (BW) |
|---|---|---|---|
| Age (yr) | 26 | 27 | 26 |
| Weight (kg) | 68.2 | 68.6 | 66.4 |
| $VO_{2max}$ ($ml \cdot kg \cdot min^{-1}$) | 55.3 | 61.5 | 52.1 |
| $VO_{2max}$ (L/min) | 3.8 | 4.2 | 3.5 |
| $V_ESTPD_{max}$ (L/min) | 118 | 114 | 114 |
| $RQ_{max}$ | 1.2 | 1.17 | 1.22 |
| $HR_{max}$ | 184 | 193 | 180 |
| $kp_{max}$ | 3.2 | 5 | 3.5 |

non-rebreathing valve, with expired gases passing into and through a 5-liter mixing chamber. $O_2$ and $CO_2$ gas fractions ($F_EO_2$, $F_ECO_2$) were analyzed by the Beckman OM-11 and LB-2, respectively, and interfaced with an Apple IIe computer. Heart rate was monitored by one-lead electrocardiography (CM5).

A crossover method of experimentation was employed, and hence each subject served as his own control. Testing was conducted at least 8 hours post-prandial, following a 12-hour period of ingestion of either placebo or 2g each of L-Aspartate, L-Ornithine, L-Arginine, and L-Glutamate. Half the dosage was consumed 12 hours before testing, with the other half consumed 1 hour before testing. Corn starch served as the placebo. A double-blind experimental design and a counterbalanced order of testing determined the order of active substance/placebo administration.

Subjects exercised to exhaustion on two occasions on a bicycle ergometer at a power output corresponding to 120% $VO_{2max}$, following placebo or amino acid ingestion.

Following a 2-minute warm-up at .75 kp, subjects pedaled at 120% $VO_{2max}$ until cadence fell below 60 rpm with best effort.

### B.2.3 Biochemical Analyses

A 5 ml blood sample was collected from a superficial forearm vein via indwelling catheter with the subject in a seated position at rest, and at 1 and 4 minutes post-exercise. Each was assayed in duplicate for blood lactate (lactate dehydrogenase method), plasma $NH_3$ (phenol hypochlorite method), and plasma urea (urease/phenol hypochlorite method) utilizing Sigma Chemical Co. (St. Louis, Mo.) diagnostic kits. Each sample was injected immediately into a green-top Vacutainer@ tube (heparinized) and immediately placed within an ice bath. One ml of the sample was then mixed with 2 ml of a cold 7% perchlorate solution, centrifuged (5000 rpm x 2 minutes), and the supernatant frozen for subsequent lactate determination. The remainder of the blood sample was centrifuged (5000 rpm x 2 minutes), and the harvested plasma frozen (-20 $C^{o}$) within 30 minutes after collection.

## B.3 Results

Results are presented in Table B.2. Blood samples (seated, resting) acquired at rest, one minute post-exercise (X1) and four minutes post-exercise (X4) showed a general trend of lowering of blood ammonia and rising of blood urea with supplementation. Time to exhaustion was not significantly different between treatments.

## B.4 Summary

Results from this pilot study indicate that a combination of aspartate, glutamate, arginine, and ornithine supplementation increases blood urea production and reduces blood ammonia concentration versus placebo, but has no effect on high-intensity short term performance. A larger sample size and isolation of these amino acids will further elucidate the clearance pattern and mechanisms of blood ammonia removal.

Table B.2. Individual plasma $NH_3$ [μM/L], blood urea nitrogen [mg/dl], and time to exhaustion (seconds) in response to placebo (P) or L-Aspartate, L-Glutamate, L-Ornithine, and L-Arginine (A) supplementation.

| Subject | P R [$NH_3$] | A R [$NH_3$] | P X1 [$NH_3$] | A X1 [$NH_3$] | P X4 [$NH_3$] | A X4 [$NH_3$] |
|---|---|---|---|---|---|---|
| 1 MC | 64 | 20 | 115 | 50 | 110 | 51 |
| 2 RM | 21 | 13 | 26 | 17 | 32 | 22 |
| 3 BW | 14 | 33 | 48 | 16 | 46 | 24 |
| | | | | | | |
| Mean | 33.0 | 22.0 | 63.0 | 27.7 | 62.7 | 32.3 |
| SEM | 15.6 | 5.9 | 26.8 | 11.2 | 24.0 | 9.4 |
| SD | 27.1 | 10.1 | 46.4 | 19.3 | 41.6 | 16.2 |
| Minimum | 14 | 13 | 26 | 16 | 32 | 22 |
| Maximum | 64 | 33 | 115 | 50 | 110 | 51 |

| Subject | P R [BUN] | A R [BUN] | P X1 [BUN] | A X1 [BUN] | P X4 [BUN] | A X4 BUN] |
|---|---|---|---|---|---|---|
| 1 MC | 19 | 17 | 17 | 17 | 17 | 23 |
| 2 RM | 16 | 10 | 14 | 23 | 12 | 22 |
| 3 BW | 14 | 18 | 24 | 28 | 24 | 22 |
| | | | | | | |
| Mean | 16.3 | 15.0 | 18.3 | 22.7 | 17.7 | 22.3 |
| SEM | 1.5 | 2.5 | 3.0 | 3.2 | 3.5 | 0.3 |
| SD | 2.5 | 4.4 | 5.1 | 5.5 | 6.0 | 0.6 |
| Minimum | 14 | 10 | 14 | 17 | 12 | 22 |
| Maximum | 19 | 18 | 24 | 28 | 24 | 23 |

| Subject | P WTE | A WTE |
|---|---|---|
| 1 MC | 89.9 | 82.4 |
| 2 RM | 171.4 | 178.4 |
| 3 BW | 154.5 | 148 |
| | | |
| Mean | 138.6 | 136.3 |
| SEM | 24.8 | 28.3 |
| SD | 43.0 | 49.1 |
| Minimum | 89.9 | 82.4 |
| Maximum | 171.4 | 178.4 |

P = placebo; A = amino acid supplementation; R = at rest; X1 = 1 minute post-exercise; X4 = 4 minutes post-exercise; $NH_3$ = plasma ammonia concentration [μM/L]; BUN = blood urea nitrogen [mg/dl]; SEM = standard error of measurement; SD = standard deviation from the mean; WTE = work time to exhaustion (seconds).

# APPENDIX C. PILOT STUDY 2: THE EFFECTS OF L-ARGININE AND L-ORNITHINE SUPPLEMENTATION ON AMMONIA CLEARANCE AND EXERCISE PERFORMANCE

## C.1 Introduction

The non-essential non-toxic amino acids L-Arginine (L-Arg) and L-Ornithine (L-Orn) clinically attenuate hyperammonemia associated with hepatic encephalopathy (Fahey, 1957; Salvatore et al., 1964; Batshaw, 1984). Pilot work in our laboratory has also found sharp drops in blood ammonia and rise in blood urea nitrogen in response to short-term high-intensity cycle ergometry with supplementation of ammonia reducing amino acids including L-Arginine and L-Ornithine (i.e. aspartate, glutamate, arginine, ornithine). However, due to varying biochemical pathways, it is unclear as to the magnitude of the effect contributed by the different types of amino acids. Further, to date there are no published scientific reports as to supplementation of the ammonia-clearing urea cycle amino acids L-Arginine and L-Ornithine to improve 1) exercise-induced hyperammonemia, 2) time to exhaustion, and 3) urea cycle activity. Therefore, the purpose of this pilot study was to isolate and explore the effects of L-Arginine and L-Ornithine supplementation on blood ammonia and urea, and time to exhaustion with ramp treadmill exercise.

## C.2 Methods

Ten moderately-trained ($VO_{2max}$ 52.2 ml/kg/min ± 2.2 SEM) college males exercised to exhaustion on two occasions, using a modified Bruce protocol. Subject characteristics are presented in Table C.2. $VO_{2max}$ was measured using a Sensormedics 2900 metabolic cart. Subjects ingested either 10g L-Arginine and 10g L-Ornithine (A), or placebo (P), administered 12 hours and 30' before the treadmill test. Forearm venous samples were taken at 0, 1, 3, and 5 minutes during seated recovery, and centrifuged within one minute of collection. Plasma samples were placed into an ice bath and assayed within five minutes for urea nitrogen [BUN] and [$NH_3$] by a Kodak Ektachem DT60 analyzer.

Table C.1. Descriptive characteristics of subjects in pilot study 2.

| Subject | Age (yr) | Weight (kg) | $VO_{2max}$ ($ml \cdot kg^{-1} \cdot min^{-1}$) |
|---|---|---|---|
| 1 | 18 | 75 | 56.5 |
| 2 | 23 | 74 | 49.2 |
| 3 | 25 | 75 | 51.1 |
| 4 | 22 | 65 | 56.7 |
| 5 | 22 | 102 | 39.0 |
| 6 | 25 | 79 | 54.9 |
| 7 | 23 | 90 | 43.5 |
| 8 | 20 | 75 | 60.5 |
| 9 | 21 | 68 | 58.8 |
| 10 | 21 | 61 | 51.3 |
| Mean | 22.0 | 76.4 | 52.2 |
| SEM | 0.7 | 3.8 | 2.2 |
| SD | 2.2 | 12.0 | 6.8 |
| Minimum | 18.0 | 61.0 | 39.0 |
| Maximum | 25.0 | 102.0 | 60.5 |

SEM – standard error of measurement; SD – standard deviation from the mean

**C.3 Results**

Table C.2 presents individual BUN responses [mg/dl] to 10g L-Arginine and 10g L-Ornithine (A) and placebo (P), while Table C.3 presents individual blood ammonia responses [μM/L], respectively. [BUN] was ~ 40% higher in the A group vs. placebo during recovery. Blood [$NH_3$] continued to rise after exercise, but did not differ between treatment groups. Time to exhaustion and $VO_{2max}$ did not differ across treatments (10.63 minutes, A; vs. 10.5 minutes, P).

**C.4 Summary**

This study was designed to examine the effects of L-Arginine and L-Ornithine supplementation on ammonia metabolism and urea cycle activity following maximal exercise. We found a large significant increase in the output of urea following L-Arginine and L-Ornithine supplementation; however, a similar change in blood ammonia reduction

Table C.2. Individual blood urea nitrogen [mg/dl] at rest (R), exhaustion (X0), and during recovery (X1, X3, and X5) in response to placebo (P) or 10g L-Arginine and 10g L-Ornithine (A) supplementation. Complete bloodwork was available for n = 8.

| Subject | P R [BUN] | P X0 [BUN] | P X1 [BUN] | P X3 [BUN] | P X5 [BUN] |
|---|---|---|---|---|---|
| 1 | 11 | 11 | 12 | 12 | 11 |
| 2 | 11 | 11 | 12 | 11 | 10 |
| 3 | 11 | 11 | 10 | 10 | 10 |
| 4 | 12 | 11 | 9 | 11 | 10 |
| 5 | 10 | 9 | 11 | 9 | 9 |
| 6 | 12 | 10 | 11 | 10 | 10 |
| 7 | 12 | 11 | 12 | 10 | 10 |
| 8 | 11 | 11 | 11 | 10 | 10 |
| Mean | 11.3 | 10.6 | 11.0 | 10.4 | 10.0 |
| SEM | 0.2 | 0.3 | 0.4 | 0.3 | 0.2 |
| SD | 0.7 | 0.7 | 1.1 | 0.9 | 0.5 |
| Minimum | 10 | 9 | 9 | 9 | 9 |
| Maximum | 12 | 11 | 12 | 12 | 11 |

| Subject | A R [BUN] | A X0 [BUN] | A X1 [BUN] | A X3 [BUN] | A X5 [BUN] |
|---|---|---|---|---|---|
| 1 | 17 | 16 | 15 | 16 | 15 |
| 2 | 13 | 11 | 11 | 11 | 11 |
| 3 | 12 | 12 | 11 | 11 | 11 |
| 4 | 19 | 17 | 17 | 17 | 17 |
| 5 | 12 | 16 | 15 | 15 | 14 |
| 6 | 18 | 16 | 16 | 16 | 16 |
| 7 | 18 | 18 | 17 | 16 | 16 |
| 8 | 16 | 15 | 15 | 15 | 14 |
| Mean | 15.6 | 15.1 | 14.6 | 14.6 | 14.3 |
| SEM | 1.0 | 0.9 | 0.8 | 0.8 | 0.8 |
| SD | 2.9 | 2.4 | 2.4 | 2.3 | 2.2 |
| Minimum | 12 | 11 | 11 | 11 | 11 |
| Maximum | 19 | 18 | 17 | 17 | 17 |

P = placebo; A = amino acid supplementation; X0 = exhaustion; X1 = 1 minute post-exercise; X3 = 3 minutes post-exercise; X5 = 5 minutes post-exercise; BUN = plasma urea nitrogen [mg/dl]; SEM – standard error of measurement; SD – standard deviation from the mean

Table C.3. Individual blood $NH_3$ [μM/L] at rest (R), exhaustion (X0), and during recovery (X1, X3, and X5) in response to placebo (P) or 10g L-Arginine and 10g L-Ornithine (A) supplementation. Complete bloodwork was available for n = 7.

| Subject | P R [$NH_3$] | P X0 [$NH_3$] | P X1 [$NH_3$] | P X3 [$NH_3$] | P X5 [$NH_3$] |
|---|---|---|---|---|---|
| 1 | 46 | 152 | 171 | 169 | 155 |
| 2 | 57 | 188 | 191 | 196 | 281 |
| 3 | 78 | 163 | 186 | 279 | 221 |
| 4 | 71 | 165 | 241 | 244 | 227 |
| 5 | 87 | 206 | 215 | 199 | 283 |
| 6 | 59 | 206 | 228 | 250 | 282 |
| 7 | 125 | 255 | 261 | 277 | 208 |
| | | | | | |
| Mean | 74.7 | 190.7 | 213.3 | 230.6 | 236.7 |
| SEM | 9.9 | 13.4 | 12.2 | 16.2 | 18.3 |
| SD | 26.1 | 35.4 | 32.4 | 42.9 | 48.3 |
| Minimum | 46 | 152 | 171 | 169 | 155 |
| Maximum | 125 | 255 | 261 | 279 | 283 |

| Subject | A R [$NH_3$] | A X0 [$NH_3$] | A X1 [$NH_3$] | A X3 [$NH_3$] | A X5 [$NH_3$] |
|---|---|---|---|---|---|
| 1 | 78 | 105 | 145 | 130 | 123 |
| 2 | 70 | 135 | 142 | 112 | 140 |
| 3 | 47 | 193 | 209 | 233 | 239 |
| 4 | 56 | 178 | 209 | 267 | 255 |
| 5 | 49 | 195 | 222 | 224 | 248 |
| 6 | 91 | 189 | 217 | 233 | 270 |
| 7 | 112 | 196 | 214 | 255 | 190 |
| | | | | | |
| Mean | 71.9 | 170.1 | 194.0 | 207.7 | 209.3 |
| SEM | 9.0 | 13.5 | 13.2 | 23.1 | 22.2 |
| SD | 23.8 | 35.8 | 34.8 | 61.2 | 58.8 |
| Minimum | 47 | 105 | 142 | 112 | 123 |
| Maximum | 112 | 196 | 222 | 267 | 270 |

P = placebo; A = amino acid supplementation; X0 = exhaustion; X1 = 1 minute post-exercise; X3 = 3 minutes post-exercise; X5 = 5 minutes post-exercise; $NH_3$ = blood ammonia concentration [μM/L]; SEM – standard error of measurement; SD – standard deviation from the mean

was not observed. The pattern for the attenuation of exercise-induced hyperammonemia varies. Some studies have shown that ammonia clearance is rapid following exercise (Banister et al., 1983; Banister et al., 1985), while others have indicated that blood ammonia levels continue to rise after intense exercise (Wilkerson et al., 1975; Wilkerson et al., 1977). Several studies have suggested that there are a number of factors that regulate ammonia clearance during recovery including muscle fiber type, training state, exercise mode, and terminating intensity (Graham et al., 1995; Graham et al., 1995a).

Under normal, resting conditions, the majority of ammonia produced is excreted as urea, the principal nitrogen component of urine. When the urea cycle is stressed during intense muscular work, accessory pathways (i.e. glutamate + $NH_3$ <=> glutamine) are detected. In addition, an accelerated conversion of ammonia into carbamoyl phosphate for entry into the urea cycle increases pyrimidine by-products. Both glutamine and pyrimidine are present in urine.

Ammonia is a factor in promoting fatigue. However, the therapeutic impact and regime for short-term ammonia reduction during exercise is not clear. L-Glutamate, L-Arginine, and L-Ornithine have been administered to humans at rest with sharp drops in [$NH_3$] (Salvatore et al., 1964; Fahey, 1957). However, these results were in clinical situations, and thus a crossover effect to healthy, and highly-trained subjects, is questionable. Though it appears from this study that 10g of L-Arg and 10g of L-Orn sharply elevate blood urea nitrogen, they did not attenuate exercise-induced hyperammonemia nor affect work time to exhaustion.

Results from this study indicate:

1) Supplementation with urea cycle intermediates is effective for short-term urea production from exercise-induced hyperammonemia,
2) Blood [$NH_3$] levels are not affected in the same magnitude as urea production with augmented short-term ureagenesis

3) Short-term work time to exhaustion and $VO_{2max}$ on the treadmill are not enhanced by accelerated ureagenesis and
4) Additional ammonia metabolic pathways (i.e. synthesis of glutamine, alanine, hippurate, phenylacetylglutamine) clear blood [$NH_3$] in response to intense exercise.

Results from the preceding two pilot studies raised the following questions:

1) Does a short-term reduction in blood [$NH_3$] affect high-intensity physical performance indices (peak power); and, if so, what percentage of ammonia reduction ([μM/L]) is required?

2) Is there a crossover effect of blood ammonia clearance, in regard to training status across testing modalities (arm ergometry vs. leg extension) in vivo?

3) Is there a dose-response effect between variable ingestion (0,5,10,15g) of aspartate, blood [$NH_3$] at exhaustion, and maximal physical performance indices?

4) Is there an inverse relationship between maximal performance indices and blood [$NH_3$] at exhaustion, with state of training?

5) Is there an 'ammonia tolerance' threshold, i.e. an absolute blood concentration or percentage change from rest, where the subject requests to stop?

6) Do subjects have different 'ammonia tolerance' threshold(s), for arms vs. legs? Alternatively, is ammonia tolerance more reflective of status of specificity of training?

To continue the investigation of these considerations, pilot study 3 was conducted.

# APPENDIX D. PILOT STUDY 3: THE EFFECTS OF VARIABLE AMOUNTS OF L-ASPARTIC ACID ON BLOOD AMMONIA WITH HIGH-INTENSITY ARM WORK IN SURFERS

## D.1 Introduction

Results from previous investigations and work in our laboratory (Edwards et al. MSSE; Edwards et al. MSSE 32(5): S329), indicate that supplementation of 10g each of L-Arg and L-Orn sharply elevates blood urea nitrogen in response to intense short-term exercise. However, in contrast to hypotheses in this study, neither short-term exercise-induced hyperammonemia nor work time to exhaustion were significantly affected. Therefore, the metabolic and performance improvements reported in previous studies implicate isolation of the ingestion of L-Aspartic Acid. In addition, it is well known (Graham et al. MSSE 29:646) that training status affect the blood ammonia and performance response to exercise. However, it is unclear as to the responsiveness of training status to L-Aspartic Acid ingestion. Therefore, the purpose of this pilot study was twofold: 1) to assess the dose-response relationship between 0,5,10, or 15g aspartate intake and blood ammonia concentration in a testing modality (arm ergometry) trained group (surfers), and 2) of these amounts, determine the dose most likely to lower blood ammonia to improve performance.

## D.2 Methods

Four healthy well-trained college-age male surfers volunteered for the study. They were informed of the nature of the study and the risks involved before giving their consent in accordance with university guidelines. Subjects reported for five consecutive days of exercise testing, and instructed to keep diet consistent, and abstain from caffeine, tobacco, and alcohol. Descriptive characteristics of the subjects are presented in Table D.1.

Subjects reported to the lab for five consecutive days of exercise testing utilizing a seated incremental seated arm ergometry (Monark Model #881) protocol to exhaustion.

Table D.1. Descriptive characteristics of subjects in pilot study 3.

| Initials (Subject) | Age (yr) | Weight (kg) | Height (cm) | Years Surfing | W·min$^{-1}$ @ Exh | WTE (sec) |
|---|---|---|---|---|---|---|
| MA (a) | 23 | 76.4 | 177.8 | 0.5 | 225 | 249 |
| JA (b) | 25 | 75 | 175.3 | 7 | 250 | 246 |
| DA (c) | 24 | 79.5 | 185.4 | 3 | 225 | 272 |
| MI (d) | 22 | 84.1 | 177.8 | 4 | 225 | 257 |
| | | | | | | |
| Mean | 23.5 | 78.8 | 179.1 | 3.6 | 231.3 | 256.0 |
| SEM | 0.6 | 2.0 | 2.2 | 1.3 | 6.3 | 5.8 |
| SD | 1.3 | 4.0 | 4.4 | 2.7 | 12.5 | 11.6 |
| Minimum | 22 | 75 | 175.3 | 0.5 | 225 | 246 |
| Maximum | 25 | 84.1 | 185.4 | 7 | 250 | 272 |

WTE – time to exhaustion; SEM – standard error of measurement; SD – standard deviation from the mean

The test began at an initial intensity of 25 W·min$^{-1}$ at 100 rpm and increased 25 W·min$^{-1}$ every 30 seconds until exhaustion. Total time and terminal watt rate achieved per subject on session 1 was used for all sessions. Session(s) 1 and 2 subjects consumed 0 grams L-Aspartic Acid, while on session(s) 3, 4, and 5, subjects ingested 5, 10, or 15g L-aspartate (Source Naturals, Inc, Scotts Valley, CA) in counterbalanced order. The L-Aspartate was dissolved in a commercial performance beverage and divided for consumption 23 hours and 1 hour before testing.

Blood was drawn from a forearm vein in a seated position at rest and exhaustion. Samples were centrifuged immediately and the supernatant analyzed immediately for ammonia and lactate. Ammonia levels were determined using a Kodak (Eastman Kodak Company, Rochester, NY) Ektachem $NH_3$/Amon Clinical Chemistry Slide Analyzer (Johnson and Johnson Clinical Diagnostics, Rochester, NY). The procedure employs a multilayered analytical system fixed onto a polyester support. A 10 microliter plasma sample is added to a reagent layer consisting of a pH 9.2 buffer which effectively converts the ammonium ion ($NH_4^+$) to ammonia ($NH_3$). Uncharged, ammonia then passes through a

semipermeable membrane where it reacts with bromophenol blue to produce a blue dye which is read at a wavelength of 600 nanometers. The amount of ammonia present in the sample is proportional to the amount of blue dye produced. Control assays were conducted on dilutions of freeze-dried serum supplied by the company. Blood lactate was analyzed via the Accusport@ Portable Analyzer.

**D.3 Results**

Complete bloodwork was available for three of the four subjects. Data from the three sessions completed by subject 4 are included in the analyses. Individual responses by day, and by dose, are presented in Table(s) D.2 and D.3, respectively. Blood $NH_3$ [μM/L] responses at exhaustion relative to 0, 5, 10, and 15 g of L-Aspartic Acid (A) supplementation are presented in Table D.4. Subjects reached high levels of exercise-induced hyperammonemia and lactic acidosis (mean ± SE) 197.7 ± 10.8 [μM/L], and 12.2 ± 0.3 [mM]. Further, as shown in Table D.4, there was a general trend for lowering of blood ammonia with increasing dose of L-Aspartic Acid, with no effect on lactate.

**D.4 Summary**

Results from this study suggest: 1) exercise hyperammonemia is attenuated by aspartate in a dose dependent manner, and 2) there is a tapering off of hyperammonemia reduction between 10 g and 15 g of aspartate, 3) there is a possible storage effect of daily aspartate supplementation, and 4) hyperammonemia is reduced without a concomitant change in lactate.

Table D.2. Individual blood $NH_3$ [μM/L] and blood lactate [mM] responses at rest (R) and exhaustion (X) across five exercise sessions.

| Session/Sub | R [$NH_3$] | X [$NH_3$] | R [LA] | X [LA] |
|---|---|---|---|---|
| 1a | 12 | 258 | 2.3 | 11.2 |
| 1b | 30 | 207 | 2.5 | 13.2 |
| 1c | 22 | 231 | 2.5 | 11.5 |
| 1d | 18 | 249 | 2.4 | 12.2 |
| 2a | 18 | 235 | 2.6 | 12.6 |
| 2b | 37 | 237 | 2.3 | 13.7 |
| 2c | 28 | 231 | 2.7 | 13.3 |
| 2d | 20 | 259 | 3.0 | 13.3 |
| 3a | 14 | 227 | 2.7 | 11.8 |
| 3b | 18 | 218 | 2.4 | 13.7 |
| 3c | 26 | 175 | 2.5 | 12.7 |
| 3d | 16 | 250 | 2.7 | 12.2 |
| 4a | 13 | 170 | 2.2 | 12.2 |
| 4b | 20 | 212 | 2.4 | 13.7 |
| 4c | 25 | 146 | 2.5 | 11.4 |
| 5a | 21 | 139 | 2.2 | 12.2 |
| 5b | 29 | 143 | 2.8 | 9.3 |
| 5c | 18 | 137 | 2.6 | 11.2 |
| Mean | 21.4 | 206.9 | 2.5 | 12.3 |
| SEM | 1.6 | 10.2 | 0.1 | 0.3 |
| SD | 6.6 | 43.4 | 0.2 | 1.1 |
| Minimum | 12 | 137 | 2.2 | 9.3 |
| Maximum | 37 | 259 | 3 | 13.7 |

LA – blood lactate [mM]; SEM – standard error of measurement; SD – standard deviation from the mean.

Table D.3. Individual blood $NH_3$ [μM/L] and blood lactate [mM] responses at rest (R) and at exhaustion (X) with 0, 0, 5, 10, and 15 g of L-Aspartic Acid (A) supplementation.

| A (grams) | Session/Sub | R [$NH_3$] | X [$NH_3$] | R [LA] | X [LA] |
|---|---|---|---|---|---|
| 0 | 1a | 12 | 258 | 2.3 | 11.2 |
| 0 | 1b | 30 | 207 | 2.5 | 13.2 |
| 0 | 1c | 22 | 231 | 2.5 | 11.5 |
| 0 | 1d | 18 | 249 | 2.4 | 12.2 |
| 0 | 2a | 18 | 235 | 2.6 | 12.6 |
| 0 | 2b | 37 | 237 | 2.3 | 13.7 |
| 0 | 2c | 28 | 204 | 2.7 | 13.3 |
| 0 | 2d | 20 | 259 | 3.0 | 13.3 |
| 5 | 3a | 14 | 227 | 2.7 | 11.8 |
| 5 | 4b | 20 | 212 | 2.4 | 11.8 |
| 5 | 3c | 26 | 175 | 2.5 | 12.7 |
| 10 | 4a | 13 | 170 | 2.2 | 10.2 |
| 10 | 5b | 29 | 143 | 2.8 | 9.3 |
| 10 | 5c | 18 | 137 | 2.6 | 11.2 |
| 10 | 3d | 16 | 250 | 2.7 | 12.2 |
| 15 | 5a | 21 | 139 | 2.2 | 12.3 |
| 15 | 3b | 18 | 218 | 2.4 | 13.7 |
| 15 | 4c | 25 | 146 | 2.5 | 11.4 |

A – L-Aspartic Acid; Sub – Subject; R – rest; X – exhaustion; LA – blood lactate [mM]

Table D.4. Blood $NH_3$ [μM/L] at exhaustion in response to 0, 5, 10, and 15 g of L-Aspartic Acid (A) supplementation.

| Subject | 0g | 0g | 5g | 10g | 15g |
|---|---|---|---|---|---|
| a | 258 | 235 | 227 | 170 | 139 |
| b | 207 | 237 | 212 | 143 | 218 |
| c | 231 | 204 | 175 | 137 | 146 |
| d | 249 | 259 | | 250 | |
| | | | | | |
| Mean | 236.3 | 233.8 | 204.7 | 175.0 | 167.7 |
| SEM | 11.3 | 11.3 | 15.5 | 26.0 | 25.2 |
| SD | 22.5 | 22.6 | 26.8 | 52.0 | 43.7 |
| Minimum | 207 | 204 | 175 | 137 | 139 |
| Maximum | 258 | 259 | 227 | 250 | 218 |

SEM – standard error of measurement; SD – standard deviation from the mean.

APPENDIX E. RAW DATA AND ANALYSES FOR EXPERIMENT 1

Table E.1. Staff instructions for distributing placebo (P) or L-Aspartic Acid (A) packets to subjects for experiment 1 (reliability).

1) Subjects report to staff for receiving powder packets at least 24 hours prior to each exercise session
2) Staff hands out 2 packets to each subject
3) Staff instructs subject to mix one packet in 8 oz water 24 hours prior to testing, and drink, and likewise the second packet 1 one hour prior to testing.
4) Staff crosses out the A x 2, or P x 2 on the master sheet below, as packets are distributed:

| Subject | Session 1 | Session 2 | Session 3 | Session 4 | Session 5 |
|---|---|---|---|---|---|
| | A x 2 | P x 2 | A x 2 | P x 2 | P x 2 |
| | P x 2 | A x 2 | P x 2 | A x 2 | P x 2 |
| | P x 2 | P x 2 | A x 2 | P x 2 | A x 2 |
| | A x 2 | P x 2 | A x 2 | P x 2 | P x 2 |
| | P x 2 | A x 2 | P x 2 | A x 2 | P x 2 |
| | P x 2 | P x 2 | A x 2 | P x 2 | A x 2 |
| | A x 2 | P x 2 | A x 2 | P x 2 | P x 2 |
| | P x 2 | A x 2 | P x 2 | A x 2 | P x 2 |
| | P x 2 | P x 2 | A x 2 | P x 2 | A x 2 |
| | A x 2 | P x 2 | A x 2 | P x 2 | P x 2 |
| | P x 2 | A x 2 | P x 2 | A x 2 | P x 2 |
| | P x 2 | P x 2 | A x 2 | P x 2 | A x 2 |
| | A x 2 | P x 2 | A x 2 | P x 2 | P x 2 |

Table E.2. Individual blood $NH_3$ [μM/L] responses across three sessions of placebo (P1, P2, P3). Blood was drawn 3 minutes post-exercise.

| **Subject** | **P1 [μM/L]** | **P2 [μM/L]** | **P3 [μM/L]** |
|---|---|---|---|
| 1 | 179 | 211 | 199 |
| 2 | 182 | 230 | 235 |
| 3 | 151 | 139 | 138 |
| 4 | 190 | 183 | 194 |
| 5 | 91 | 107 | 88 |
| 6 | 146 | 131 | 143 |
| 7 | 134 | 150 | 163 |
| 8 | 120 | 132 | 116 |
| 9 | 256 | 293 | 237 |
| 10 | 168 | 158 | 166 |
| 11 | 207 | 219 | 189 |
| 12 | 247 | 232 | 257 |
| 13 | 259 | 276 | 230 |
| 14 | 171 | 193 | 183 |
| 15 | 193 | 200 | 225 |

| | | | |
|---|---|---|---|
| Mean | 179.6 | 190.3 | 184.2 |
| SEM | 12.6 | 14.1 | 12.6 |
| SD | 48.8 | 54.6 | 48.8 |
| Minimum | 91 | 107 | 88 |
| Maximum | 259 | 293 | 257 |

SEM – standard error of measurement; SD – standard deviation from the mean

Table E.3. Individual blood $NH_3$ [μM/L] responses across two sessions of L-Aspartic Acid supplementation (A1, A2). Blood was drawn 3 minutes post-exercise.

| **Subject** | **A1 [μM/L]** | **A2 [μM/L]** |
|---|---|---|
| 1 | 167 | 201 |
| 2 | 162 | 151 |
| 3 | 177 | 168 |
| 4 | 124 | 137 |
| 5 | 124 | 139 |
| 6 | 98 | 85 |
| 7 | 221 | 246 |
| 8 | 158 | 150 |
| 9 | 239 | 162 |
| 10 | 177 | 187 |
| | | |
| Mean | 164.7 | 162.6 |
| SEM | 13.7 | 13.6 |
| SD | 43.2 | 42.9 |
| Minimum | 98 | 85 |
| Maximum | 239 | 246 |

SEM – standard error of measurement; SD – standard deviation from the mean

Table E.4. Reliability analyses of blood $NH_3$ [μM/L] across placebo sessions (P1, P2, and P3).

RELIABILITY ANALYSIS

| | Mean | Std Dev | Cases |
|---|---|---|---|
| P1 $NH_3$ | 179.6000 | 48.8186 | 15.0 |
| P2 $NH_3$ | 190.4667 | 55.0271 | 15.0 |
| P3 $NH_3$ | 184.2000 | 48.7621 | 15.0 |

Inter-item Intraclass Correlation Coefficient Matrix

| | P1 $NH_3$ | P2 $NH_3$ | P3 $NH_3$ |
|---|---|---|---|
| P1 $NH_3$ | 1.0000 | | |
| P2 $NH_3$ | . 9109 | 1.0000 | |
| P3 $NH_3$ | . 9009 | . 8874 | 1.0000 |

Intraclass Correlation Coefficient (P1 $NH_3$ vs. P2 $NH_3$ vs. P3 $NH_3$)

One-way random effect model: People Effect Random
Single Measure Intraclass Correlation = .8998
95.00% C.I.: Lower = .7850 Upper = .9617
F = 27.9417 DF = (14, 30.0) Sig. = 0.0000 (Test Value = 0.0000 )

Intraclass Correlation Coefficient (P1 $NH_3$ vs. P2 $NH_3$)

One-way random effect model: People Effect Random
Single Measure Intraclass Correlation = .9109
95.00% C.I.: Lower = .7624 Upper = .9689
F = 21.4481 DF = (14, 15.0) Sig. = 0.0000 (Test Value = 0.0000 )

Intraclass Correlation Coefficient (P1 $NH_3$ vs. P3 $NH_3$)

One-way random effect model: People Effect Random
Single Measure Intraclass Correlation = .9009
95.00% C.I.: Lower = .7381 Upper = .9653
F = 19.1875 DF = (14, 15.0) Sig. = 0.0000 (Test Value = 0.0000 )

Intraclass Correlation Coefficient (P2 $NH_3$ vs. P3 $NH_3$)

One-way random effect model: People Effect Random
Single Measure Intraclass Correlation = .8874
95.00% C.I.: Lower = .7057 Upper = .9603
F = 16.7563 DF = (14, 15.0) Sig. = 0.0000 (Test Value = 0.0000 )

Table E.5. Reliability analysis of blood $NH_3$ [μM/L] across L-Aspartic Acid supplementation sessions (A1, A2).

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RELIABILITY ANALYSIS

Intraclass Correlation Coefficient Matrix

| | A1 $NH_3$ | A2 $NH_3$ |
|---|---|---|
| A1 $NH_3$ | 1.0000 | |
| A2 $NH_3$ | .7426 | 1.0000 |

N of Cases = 10.0

Intraclass Correlation Coefficient

One-way random effect model: People Effect Random
Single Measure Intraclass Correlation = .7643
95.00% C.I.: Lower = .3290 Upper = .9348
F = 7.4847 DF = (9, 10.0) Sig. = .0021 (Test Value = .0000 )

Table E.6. Group comparisons of blood $NH_3$ [μM/L] across three sessions of placebo (P1, P2, P3) and two of L-Aspartic Acid supplementation (A1, A2).

MEAN P1 $NH_3$ [μM/L] = 179.6
VAR. P1 $NH_3$ [μM/L] = 695.1

MEAN P2 $NH_3$ [μM/L] = 190.5
VAR. P2 $NH_3$ [μM/L] = 883.1

MEAN P3 $NH_3$ [μM/L] = 184.2
VAR. P3 $NH_3$ [μM/L] = 693.5

MEAN A1 $NH_3$ [μM/L] = 164.7
VAR. A1 $NH_3$ [μM/L] = 350.3

MEAN A2 $NH_3$ [μM/L] = 162.6
VAR. A2 $NH_3$ [μM/L] = 345.5

ANOVA SUMMARY TABLE

| SOURCE | SUM SQRES | DF | MEAN SQRES | F-RATIO | P |
|---|---|---|---|---|---|
| BETWEEN GRPS | 29215.9 | 4 | 7303.0 | 25.7 | < 0.0001 |
| WITHIN SUBJ | 87806.2 | 48 | 1829.3 | | |
| ERROR | 54633.0 | 192 | 284.6 | | |
| TOTAL | 171656.2 | 244 | | | |

TUKEY'S PROTECTED T-TESTS

| | P1 $NH_3$ | P2 $NH_3$ | P3 $NH_3$ | A1 $NH_3$ | A2 $NH_3$ |
|---|---|---|---|---|---|
| P1 $NH_3$ | 0 | -1.8 | -.748 | 2.2* | 2.5 |
| P2 $NH_3$ | -1.8 | 0 | 1.089 | 3.7** | 4.0** |
| P3 $NH_3$ | -.7 | 1.08 | 0 | 2.8** | 3.1** |
| A1 $NH_3$ | 2.2* | 3.78** | 2.88** | 0 . | .3 |
| A2 $NH_3$ | 2.5* | 4.08** | 3.18** | .3 | 0 |

** $p < 0.01$ * $p < 0.05$

APPENDIX F. RAW DATA AND ANALYSES FOR EXPERIMENT 2

Table F.1. Staff instructions for distributing placebo (P) or L-Aspartic Acid (A) supplementation packets to subjects for experiment 2 (performance) in intercollegiate water polo players (WP) and moderately-trained males (MOD).

1) Subject report to staff for receiving powder packets at least 24 hours prior to each exercise session
2) Staff hands out 2 packets to each subject
3) Staff instructs subject to mix one packet in 8 oz water 24 hours prior to testing, and drink, and likewise the second packet 1 one hour prior to testing.
4) Staff crosses out the A x 2, or P x 2 on the master sheet below, as packets are distributed:

| Subject | Session 1 | Session 2 |
|---|---|---|
| | A x 2 | P x 2 |
| | P x 2 | A x 2 |
| | A x 2 | P x 2 |
| | P x 2 | A x 2 |
| | A x 2 | P x 2 |
| | P x 2 | A x 2 |
| | A x 2 | P x 2 |
| | P x 2 | A x 2 |
| | A x 2 | P x 2 |
| | P x 2 | A x 2 |

Table F.2. Individual blood $NH_3$ [µM/L] in response to placebo (P) or L-Aspartic Acid (A) supplementation in intercollegiate water polo players (WP). Blood was drawn 3 minutes post-exercise.

| Subject | P [µM/L] | A [µM/L] |
|---|---|---|
| 1 | 273 | 269 |
| 2 | 195 | 163 |
| 3 | 307 | 233 |
| 4 | 277 | 202 |
| 5 | 302 | 287 |
| 6 | 268 | 212 |
| 7 | 170 | 113 |
| 8 | 207 | 213 |
| 9 | 170 | 113 |
| | | |
| Mean | 241.0 | 200.6 |
| SEM | 18.4 | 20.5 |
| SD | 55.3 | 61.5 |
| Minimum | 170 | 113 |
| Maximum | 307 | 287 |

SEM – standard error of measurement; SD – standard deviation from the mean

Table F.3. Individual blood $NH_3$ [µM/L] in response to placebo (P) or L-Aspartic Acid (A) supplementation in moderately-trained college males (MOD). Blood was drawn 3 minutes post-exercise.

| Subject | P [µM/L] | A [µM/L] |
|---|---|---|
| 1 | 230 | 193 |
| 2 | 150 | 90 |
| 3 | 231 | 166 |
| 4 | 172 | 156 |
| 5 | 243 | 222 |
| 6 | 133 | 120 |
| 7 | 233 | 128 |
| 8 | 243 | 227 |
| 9 | 342 | 368 |
| | | |
| Mean | 219.7 | 185.6 |
| SEM | 20.8 | 27.5 |
| SD | 62.4 | 82.5 |
| Minimum | 133 | 90 |
| Maximum | 342 | 368 |

SEM – standard error of measurement; SD – standard deviation from the mean

Table F.4. Individual performance power ($W \cdot min^{-1}$) in response to placebo (P) or L-Aspartic Acid (A) supplementation in intercollegiate water polo players (WP).

| Subject | P ($W \cdot min^{-1}$) | A ($W \cdot min^{-1}$) |
|---|---|---|
| 1 | 255 | 278 |
| 2 | 319 | 248 |
| 3 | 254 | 210 |
| 4 | 185 | 343 |
| 5 | 208 | 249 |
| 6 | 194 | 201 |
| 7 | 228 | 365 |
| 8 | 214 | 316 |
| 9 | 191 | 222 |
| | | |
| Mean | 227.6 | 270.2 |
| SEM | 14.3 | 19.8 |
| SD | 42.8 | 59.3 |
| Minimum | 185 | 201 |
| Maximum | 319 | 365 |

SEM – standard error of measurement; SD – standard deviation from the mean

Table F.5. Individual performance power ($W \cdot min^{-1}$) r response to placebo (P) or L-Aspartic Acid (A) supplementation in moderately-trained college males (MOD).

| Subject | P ($W \cdot min^{-1}$) | A ($W \cdot min^{-1}$) |
|---|---|---|
| 1 | 253 | 209 |
| 2 | 176 | 241 |
| 3 | 205 | 188 |
| 4 | 211 | 259 |
| 5 | 188 | 200 |
| 6 | 192 | 182 |
| 7 | 209 | 231 |
| 8 | 177 | 249 |
| 9 | 229 | 222 |
| | | |
| Mean | 204.4 | 220.1 |
| SEM | 8.4 | 9.1 |
| SD | 25.0 | 27.3 |
| Minimum | 176 | 182 |
| Maximum | 253 | 259 |

SEM – standard error of measurement; SD – standard deviation from the mean

Table F.6. Group comparisons of blood $NH_3$ [μM/L] in response to placebo (P) or L-Aspartic Acid supplementation (A) in intercollegiate water polo players (WP) and moderately-trained males (MOD).

MEAN $NH_3$ [μM/L] WP P = 241.0
VAR. $NH_3$ [μM/L] WP P = 720.0

MEAN $NH_3$ [μM/L] WP A = 200.6
VAR. $NH_3$ [μM/L] WP A = 889.4

MEAN $NH_3$ [μM/L] MOD P = 219.7
VAR. $NH_3$ [μM/L] MOD P = 914.8

MEAN $NH_3$ [μM/L] MOD A = 185.6
VAR. $NH_3$ [μM/L] MOD A = 1599.5

ANOVA SUMMARY TABLE

| SOURCE | SUM SQRES | DF | MEAN SQRES | F-RATIO | P |
|---|---|---|---|---|---|
| BETWEEN GRPS | 60539.08951 | 3 | 20179.6965 | 23.19 | < 0.0001 |
| WITHIN SUBJ | 51460.88889 | 34 | 1513.55556 | | |
| ERROR | 88747.55556 | 102 | 870.07407 | | |
| TOTAL | 200747.53395 | 139 | | | |

TUKEY'S PROTECTED T-TESTS

| | WP P | WP A | MOD P | MOD A |
|---|---|---|---|---|
| WP P | 0 | 2.90862** | 1.53422 | 3.98737** |
| WP A | 2.90862** | 0 | -1.3744 | 1.07875 |
| MOD P | 1.53422 | -1.3744 | 0 | 2.45315* |
| MOD A | 3.98737** | 1.07875 | 2.45315* | 0 |

** $p < 0.01$ * $p < 0.05$

Table F.7. Group comparisons of performance power ($W \cdot min^{-1}$) in response to placebo (P) or L-Aspartic Acid (A) supplementation in intercollegiate water polo players (WP) and moderately-trained males (MOD).

MEAN $W \cdot min^{-1}$ WP P = 227.5
VAR. $W \cdot min^{-1}$ WP P = 428.1

MEAN $W \cdot min^{-1}$ WP A = 270.3
VAR $W \cdot min^{-1}$ WP A = 827.6

MEAN $W \cdot min^{-1}$ MOD P = 204.4
VAR. $W \cdot min^{-1}$ MOD P = 148.4

MEAN $W \cdot min^{-1}$ MOD A = 220.2
VAR. $W \cdot min^{-1}$ MOD A = 175.5

ANOVA SUMMARY TABLE

| SOURCE | SUM SQRES | DF | MEAN SQRES | F RATIO | P |
|---|---|---|---|---|---|
| BETWEEN GRPS | 83426.83287 | 3 | 27808.94429 | 76.09 | < 0.0001 |
| WITHIN SUBJ | 16435.435 | 34 | 483.39515 | | |
| ERROR | 37275.59833 | 102 | 365.44704 | | |
| TOTAL | 137137.8662 | 139 | | | |

TUKEY'S PROTECTED T-TESTS

| | WP P | WP A | MOD P | MOD A |
|---|---|---|---|---|
| WP P | 0 | -4.75062** | 2.56704* | .81376 |
| WP A | -4.75062** | 0 | 7.31766** | 5.56438** |
| MOD P | 2.56704* | 7.31766** | 0 | -1.75328 |
| MOD A | .81376 | 5.56438** | -1.75328 | 0 |

** $p < 0.01$ * $p < 0.05$

## APPENDIX G. RAW DATA AND ANALYSES FOR EXPERIMENT 3

Table G.1. Staff instructions for distributing placebo (P) and L-Aspartic Acid (A) packets packet(s) to subjects for experiment 3 (performance) in recreationally-trained females (FEM).

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1) Subject report to staff for receiving powder packets at least 24 hours prior to each exercise session
2) Staff hands out 2 packets to each subject
3) Staff instructs subject to mix one packet in 8 oz water 24 hours prior to testing, and drink, and likewise the second packet 1 one hour prior to testing.
4) Staff crosses out the A x 2, or P x 2 on the master sheet below, as packets are distributed:

| **Subject** | **Session 1** | **Session 2** |
|---|---|---|
| | A x 2 | P x 2 |
| | P x 2 | A x 2 |
| | A x 2 | P x 2 |
| | P x 2 | A x 2 |
| | A x 2 | P x 2 |
| | P x 2 | A x 2 |
| | A x 2 | P x 2 |
| | P x 2 | A x 2 |
| | A x 2 | P x 2 |
| | P x 2 | A x 2 |

Table G.2. Individual blood $NH_3$ [μM/L] in response to placebo (P) or L-Aspartic Acid (A) supplementation in recreationally-trained female subjects (FEM). Blood was drawn 3 minutes post-exercise.

| Subject | P [μM/L] | A [μM/L] |
|---|---|---|
| 1 | 207 | 245 |
| 2 | 257 | 283 |
| 3 | 230 | 191 |
| 4 | 161 | 140 |
| 5 | 226 | 166 |
| 6 | 222 | 138 |
| 7 | 300 | 306 |
| 8 | 249 | 321 |
| 9 | 257 | 218 |
| 10 | 198 | 203 |
| 11 | 183 | 140 |
| 12 | 150 | 211 |
| 13 | 193 | 186 |
| 14 | 185 | 213 |
| 15 | 238 | 252 |
| 16 | 257 | 273 |
| 17 | 150 | 131 |
| 18 | 142 | 102 |
| | | |
| Mean | 215.5 | 212.8 |
| SEM | 10.3 | 14.6 |
| SD | 42.4 | 60.2 |
| Minimum | 150 | 131 |
| Maximum | 300 | 321 |

SEM – standard error of measurement; SD – standard deviation from the mean

Table G.3. Individual performance power ($W \cdot min^{-1}$) in response to placebo (P) or L-Aspartic Acid (A) supplementation in recreationally-trained female subjects (FEM).

| **Subject** | **P ($W \cdot min^{-1}$)** | **A ($W \cdot min^{-1}$)** |
|---|---|---|
| 1 | 195 | 174 |
| 2 | 199 | 209 |
| 3 | 182 | 212 |
| 4 | 171 | 124 |
| 5 | 168 | 175 |
| 6 | 138 | 154 |
| 7 | 144 | 148 |
| 8 | 140 | 196 |
| 9 | 132 | 111 |
| 10 | 272 | 168 |
| 11 | 156 | 120 |
| 12 | 135 | 170 |
| 13 | 204 | 193 |
| 14 | 146 | 164 |
| 15 | 122 | 137 |
| 16 | 171 | 167 |
| 17 | 180 | 193 |
| 18 | 194 | 277 |
| | | |
| Mean | 167.9 | 165.6 |
| SEM | 8.9 | 7.4 |
| SD | 36.7 | 30.3 |
| Minimum | 122 | 111 |
| Maximum | 272 | 212 |

SEM – standard error of measurement; SD – standard deviation from the mean

Table G.4. Group comparisons of blood $NH_3$ [μM/L] in response to placebo (P) or L-Aspartic Acid supplementation (A) in recreationally-trained female subjects (FEM).

MEAN $NH_3$ [μM/L] FEM P = 211.6
VAR. $NH_3$ [μM/L] FEM P = 1981.5

MEAN $NH_3$ [μM/L] FEM A = 206.6
VAR. $NH_3$ [μM/L] FEM A = 4088.4

ANOVA SUMMARY TABLE

| SOURCE | SUM SQRES | DF | MEAN SQRES | F-RATIO | P |
|---|---|---|---|---|---|
| BETWEEN GRPS | 225 | 1 | 225 . | 2554 | .6197 |
| WITHIN SUBJ | 88217.55556 | 17 | 5189 | | |
| ERROR | 14971 | 17 | 880.64706 | | |
| TOTAL | 103413.55556 | 35 | | | |

Table G.5. Group comparisons of performance power ($W \cdot min^{-1}$) in response to placebo (P) or L-Aspartic Acid supplementation (A) in recreationally-trained female subjects (FEM).

MEAN FEM $W \cdot min^{-1}$ P = 172.4
VAR. FEM $W \cdot min^{-1}$ P = 1235.1

MEAN FEM $W \cdot min^{-1}$ A = 179.1
VAR. FEM $W \cdot min^{-1}$ A = 1177.9

ANOVA SUMMARY TABLE

| SOURCE | SUM SQRES | DF | MEAN SQRES | F-RATIO | P |
|---|---|---|---|---|---|
| BETWEEN GRPS | 404.68028 | 1 | 404.68028 | .54354 | .471 |
| WITHIN SUBJ | 28364.41472 | 17 | 1668.49498 | | |
| ERROR | 12657.01472 | 17 | 744.53028 | | |
| TOTAL | 41426.10972 | 35 | | | |

# VITA

William W. Edwards was born on May 29, 1959, in Baton Rouge, Louisiana. He moved to Conroe, Texas at the age of eight, where he graduated from Conroe High School in 1977.

He attended New Mexico Junior College, Hobbs, New Mexico, San Jacinto Junior College - North, Houston, Texas, and Sam Houston State University, Huntsville, Texas on golf scholarships, where he was a member of two NCAA Division II national championship teams. He was awarded a Bachelor of Science from Sam Houston State University in May of 1982 (Physical Education), and another Bachelor of Science in December of 1983 (Mathematics). After two years in the restaurant business, he enrolled in the exercise physiology graduate program in the School of Health, Physical Education, Recreation, and Dance (now the Department of Kinesiology), at Louisiana State University in Baton Rouge, Louisiana. He received his Master of Science degree in Exercise Science in December of 1989.

He then worked as an exercise physiologist at The Heart and Fitness Center, Baton Rouge, Louisiana from 1989 - 1994, and Lallie Kemp Medical Center, Independence, Louisiana from 1994 – 1995. In 1995, he enrolled in the exercise science doctoral program in the Department of Kinesiology at Louisiana State University, Baton Rouge, Louisiana, to pursue a degree in exercise physiology and nutrition.